REMARKS/ARGUMENTS

Claims 1, 8-40, and 46-67 are currently pending and under examination in the application.

Claim 1 has been amended herein to incorporate the limitation of claim 2 (reciting SAHA specifically) and claim 2 has been canceled. Claim 24 has been amended to incorporate the limitation of claim 25, reciting oral administration, and claim 25 has been canceled. Claims 26, 30, 31 and 40 have been amended to correct claim dependency in view of the amendment to claim 24. No new matter has been added.

Claims 3-7 and 41-45 were previously canceled as directed to non-elected subject matter. Applicants reserve the right to present this subject matter in a co-pending application.

35 U.S.C. §132

The Examiner maintains the objection to the recitation of "and/or the anti-cancer agents," indicating that the phrase introduces new matter with respect to the word "or." Applicants reiterate that this phrase is supported by the application as originally filed (see, *inter alia*, page 16, line 19 through page 17, line 3 (discussing administration of any of a number of various anti-tumor agents to a subject with neoplastic cells), and page 46, lines 21-26 of the application (discussing that the compounds of the invention, or hydrates thereof, can be incorporated into pharmaceutical compositions).

Reconsideration and withdrawal of this objection is respectfully requested.

35 U.S.C. §103(a)

There is a sole remaining rejection in this application -- rejection of claims 1, 2, 8-40, and 46-67 are unpatentable over Jackson under 35 U.S.C. §103(a). The Examiner

indicates that based on Jackson, one of skill in the art would have been motivated to treat mesothelioma by the administration of SAHA, and further contends that the determination of an optimal dosing regimen involves no more than routine experimentation.

Applicants disagree. A rejection under § 103 cannot be predicated on the mere identification of individual components of claimed inventions in the prior art. Rather, to make out an obviousness rejection, there must be a teaching or suggestion that motivates the ordinarily skilled artisan to select the specifically claimed invention, and there must be a reasonable expectation of success in the prior art. This is lacking here.

Jackson does no more than provide two laundry lists. One laundry list is of HDAC inhibitors, including trichostatin A, trapoxin A, MS-275, CHAPs, CI-944, SAHA, depsipeptide, CBHA, pyroxamide, CHAP31, HC-toxin, chlamydocin, Cly-2, WF-3161, Tan-1746, apicidin, and analogs thereof. The other laundry list is a plethora of cancers (over 160 types), only one of which is mesothelioma. However, there is nothing in Jackson to teach or suggest the desirability of making the specific combination of using SAHA (or salts or hydrates thereof) to treat mesothelioma, as claimed here. At best, Jackson provides a general disclosure which, although may generally pique the scientist's curiosity for further investigation to try an HDAC inhibitor with a cancer type (*i.e.*, "obvious-to-try), does not provide sufficient teaching of how to obtain the desired result (*i.e.*, treating mesothelioma with SAHA), or any reasonable expectation of success that the desired result would be obtained. "Obvious-to-try" is not the standard under § 103. *See In re Eli Lilly & Co.*, 902 F.2d 943, 14 USPQ2d 1741, 1743 (Fed. Cir. 1990); *see also Ex parte Levengood*, 28 USPQ2d 1300,1301-02 (B.P.A.I. 1993). Nor does Jackson provide a single working example demonstrating the effectiveness of any HDAC inhibitor (let alone SAHA) in treating patients afflicted with mesothelioma. Rather, Jackson characterizes one HDAC isoform (HDAC9), and concludes that it is overexpressed in breast and prostate cancers. Accordingly, Jackson cannot be considered enabling for using hydroxamic acid derived HDAC inhibitors (and certainly not SAHA) in treating mesothelioma, and should not be considered prior art in this regard. *See*

Helifix, Ltd. V. Blok-Lok, Ltd., 208 F.3d 1339, 54 USPQ2d 1299, 1304 (Fed. Cir. 2000).

For the above reasons, a *prima facie* case of obviousness has not been established and that the rejection should be withdrawn. Even if a *prima facie* case of obviousness has been made (and it has not), there is conclusive evidence of secondary considerations that supports a conclusion of nonobviousness here.

First, mesothelioma is a particularly difficult cancer to treat. See, e.g., van Ruth et al., Chest, 123: 551-561 (Feb. 2003) ("van Ruth") (copy enclosed) which identifies mesothelioma as a particularly difficult cancer to treat, with no commercially available treatment strategies. *See* van Ruth, Abstract and Discussion. Untreated, the median survival time is less than one year. Chemotherapy agents that have shown great promise in other cancers have been shown ineffective in treatment of mesothelioma. DNA intercalating agents such as mitoxantrone, menogaril, amsacrine and diaziquone have shown almost no clinical activity against mesothelioma. Ryan et al., Chest, 113; pp. 66S-73S, p. 67S (1998) ("Ryan 1998") (copy enclosed). Likewise, vinca alkaloids such as vindesine, vinblastine, vincristine, etoposide, and paclitaxel have produced little or no effect in mesothelioma. Ryan 1998, p. 67S-68S, Table 4. Alkylating agents such as ifosamide and cyclophosphamide have shown only minor activity or no activity. Ryan 1998, p. 68S, Table 4. Antimetabolites such as acivicin, dideazafolic acid, and fluorouracil are largely ineffective. Ryan 1998, p. 70S, Table 4. Further, the anthracyclines epirubicin and zorubicin given together with ifosamide and dacarbazine, respectively, have shown poor responses. Ryan 1998 p. 70S, Table 4.

Van Ruth and Ryan 1998 illustrate the long felt but unresolved need to identify an effective therapy for treating mesothelioma. Applicants claimed invention satisfies this long felt yet unresolved need. *See* specification, Example 5 and Figure 13 which provides data supporting the conclusion that SAHA is effective at treating mesothelioma tumors in patients.

Second, others have tried, and failed, to identify a suitable HDAC inhibitor for effectively treating mesothelioma. Ryan et al., J. Clin. Onc., 23(17): 3912-3922 (June

2005) ("Ryan 2005") (copy enclosed) details a study of the HDAC inhibitor MS-275 (a pyridal carbamate HDAC inhibitor) in patients with solid tumors. Ryan 2005 concludes that the oral formulation of MS-275 at the dose and schedule explored was intolerable. *See* Ryan 2005, Abstract. One of thirty-one patients in the study had mesothelioma. *See id.* at 3915, Table 1. Ryan 2005 further concludes that the development of an oral HDAC inhibitor will be a challenge. *See id.* at 3919, col. 2. Yet, this is precisely what applicants have done with SAHA. Ryan 2005 rebuts the assertion that any HDAC inhibitor from the laundry list in Jackson can be effectively used to treat mesothelioma -- as Ryan 2005 demonstrates, that is clearly not the case. This is evidence of the nonobviousness of the claimed methods. *See Timely Prods. Corps. V. Stanley Arron*, 523 F.2d 288, 187 USPQ 257, 261 (2d Cir. 1975).

Finally, SAHA is superior to other HDAC inhibitors that have been tested for treatment of mesothelioma. Kelly et al., J. Clin. Onc., 23(17): 3923-3931 (June 2005) ("Kelly") (copy enclosed) reports on clinical results using the hydroxamic acid HDAC inhibitor SAHA (according to the claimed methods). Patients enrolled in the study included those having mesothelioma. *See* Kelly at 3925, Results. Kelly demonstrates that SAHA can be administered safely for prolonged periods of time while maintaining the biologic effect of the drug and exhibiting antitumor activity at multiple dose levels and schedules (in contrast to the studies with MS-275 reported in Ryan 2005). Importantly, Kelly notes that "of particular interest" was the clinical activity observed in patients with malignant mesothelioma - two patients with partial responses (unconfirmed). *See id.* at 3930 col. 2. The results in Kelly evidence the unexpected and superior results with SAHA according to the claimed methods here.

For the foregoing reasons, and in view of the amendments to the claims, the § 103 rejection over Jackson should be withdrawn.

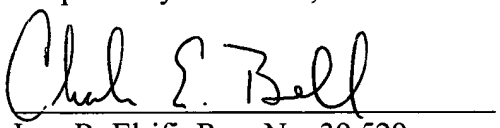
CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion of this Amendment would be deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

A petition for a two month extension of time and corresponding fee accompany this response. With the extension, the response is due by December 6, 2005. The Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. **50-0311**, Reference Number: **24852-501 CIP3**.

Date: November 15, 2005

Respectfully submitted,



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A review of chemotherapy trials for malignant mesothelioma

CW Ryan, J Herndon and NJ Vogelzang

Chest 1998;113;66-73

A Review of Chemotherapy Trials for Malignant Mesothelioma*

Christopher W. Ryan, MD; James Herndon, PhD; and Nicholas J. Vogelzang, MD

Treatment of malignant pleural mesothelioma continues to be frustrating regardless of the modality employed. Numerous trials of chemotherapeutic agents have been performed, but until recently, these studies were small and subject to inaccuracies of disease measurement. To our knowledge, no chemotherapeutic regimen has emerged as a standard of care. A review of the literature reveals that small activity against this disease has been shown by the anthracyclines, platinum compounds, and alkylating agents, whereas higher activity has been reported with the antimetabolites. The plant alkaloids have not demonstrated any activity against mesothelioma. Dose-escalated chemotherapeutic regimens may offer an advantage, whereas combination chemotherapy has not shown any benefit over single-agent therapy. Favorable responses have been reported with the administration of intrapleural biological response modifiers. Further trials and the investigation of new agents in the treatment of this disease are necessary. ***(CHEST 1998; 113:66S-73S)***

Malignant pleural mesothelioma is an aggressive and devastating malignancy, well known in the occupational medicine and legal communities. The relationship between asbestos exposure and mesothelioma was first described by Wagner et al[1] and further defined by Selikoff et al[2] in the 1960s. It is a uniformly fatal disease, with approximately 1,500 cases reported yearly in the United States.[3] Median patient age at diagnosis is 60 years, and men are affected five times as often as women.[4] Patients often present with symptoms of chest pain, dyspnea, and weight loss. Median survival for untreated patients has been 6 to 18 months in various studies.[5]

Asbestos exposure has been associated with most reported cases of mesothelioma. Asbestos is classified into two forms, the "curly" serpentines and the "needlelike" amphiboles.[6] The amphiboles include crocidolite, thought to be the most carcinogenic of asbestos fibers. Today, the most common form of asbestos in use is chrysotile, a serpentine fiber that is believed to carry a very low risk of mesothelioma but is sometimes contaminated with amphiboles. The carcinogenicity of asbestos seems to be due to the inability of phagocytic cells to digest the fiber, leading to polymorphonuclear cell activation with resultant free radical generation and mesothelial cell mutation.[7] The average time between initial asbestos exposure and diagnosis of mesothelioma is 35 to 40 years.[3]

Though much is known about the etiology of mesothelioma, treatment of the disease continues to be frustrating. Surgery, radiotherapy, chemotherapy, and multimodality approaches have all been used, as well as supportive care alone. The most aggressive surgical treatment is extrapleural pneumonectomy (EPP), for which a limited number of patients are usually eligible and which carries a contemporary operative mortality rate of 3 to 10% with only a modest improvement in overall survival.[8,9] Less than 20% of patients undergoing EPP remain free of disease at 3 years. Pleural decortication carries less risk than EPP and may help to limit recurrent pleural effusions. The role of radiotherapy seems limited to palliation, as delivering curative doses of radiation to large areas of pleura is difficult without damaging underlying lung. Use of multimodality regimens involving EPP, radiotherapy, and chemotherapy has resulted in mild improvements in median survival.[8] Intracavitary photodynamic therapy is a new approach that has been combined with surgical resection.[10] Patients receiving supportive care alone have short survival and suffer from progressive pain and pulmonary compromise as the tumor gradually encases the lung.

The role of systemic chemotherapy for malignant pleural mesothelioma continues to be an area of active investigation. Numerous agents have been tested over the years, but few have shown any clear benefit, and no regimen has emerged as a standard of care. Because of the rarity of the disease, most trials have been small and therefore lacking in statistical significance. Trials performed before the advent of CT scanning could not accurately measure disease extent and were fraught with inaccuracies. Structured, multi-institutional trials have become necessary to gather reasonable data on the use of chemotherapy in mesothelioma. The Cancer and Leukemia Group B (CALGB) began its mesothelioma program in 1984 with the hope of performing structured chemotherapy trials and pooling the resources of multiple institutions. To date, seven trials with a total of 337 eligible patients have been completed (Table 1).[11-20]

Interpretation of the data obtained using various treatment protocols for malignant pleural mesothelioma has been hindered by lack of a precise and universal staging system for the disease. The International Mesothelioma Interest Group has proposed a new tumor nodes metastases (TNM) staging system that expands on previous systems (Table 2).[5] The system incorporates new data from recent studies regarding the natural history of the disease. Systematic application of such a staging system will allow more accurate planning and precise assessment of future therapeutic trials.

Other factors that influence the survival of patients with malignant mesothelioma have been analyzed in previous studies. Tumors with epithelial histology, for example, seem to carry a better prognosis.[4,21] The CALGB has recently analyzed a number of pretreatment factors that influence survival. Data were pooled from seven phase II

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Table 1—*CALGB: Chemotherapy Trials for Malignant Mesothelioma**

Investigator(s)	CALGB Study No.	Agent(s)	No. of Patients	Year Completed
Chahinian et al[11]	8435	Mitomycin/cisplatin vs doxorubicin/cisplatin	76	1986
Vogelzang et al[12]	8638	Carboplatin	41	1988
Harmon et al[13]	8833	DHAC	41	1989
Vogelzang et al[14]	8933	Trimetrexate (6 mg/m^2/d and 10 mg/m^2/d)	51	1991
Samuels et al[15,16]	9031	DHAC/cisplatin	35	1993
Belani et al[17,18]	9131	Edatrexate and edatrexate/leucovorin	58	1994
Vogelzang et al[19]	9234	Paclitaxel/G-CSF	35	1994
Total			337	

*G-CSF=granulocyte colony-stimulating factor. Adapted from Herndon et al.[20]

studies the group has completed to date. Among the factors examined in univariate and multivariate analyses, worse prognosis was seen in those patients with a poor performance status, chest pain, dyspnea, platelet count >400,000/μL, weight loss, serum lactate dehydrogenase level >500 U/L, pleural involvement, low hemoglobin, high WBC count, age older than 75 years, and nonepithelial histology.[20] Exponential survival trees were created, and six prognostic groups were defined (Table 3).[20] The group with the best prognosis had a median survival of 13.9 months and consisted of patients aged younger than 49 years with an Eastern Cooperative Oncology Group (ECOG) performance status of 0, as well as patients aged 49 years or older with an ECOG performance status of 0 and hemoglobin ≥14.6 g/dL. The group with the worst prognosis—patients with an ECOG performance status of 1 or 2 and a WBC count ≥15.6×10^9/L—had a median survival of 1.4 months. Classifying patients by prognostic group and the new TNM staging system may help more accurately plan and interpret future phase II studies.

This review summarizes the larger chemotherapy trials for malignant pleural mesothelioma completed to date. Trials using both single-agent and combination chemotherapy are reviewed. Data from several new trials have been added since the last reviews on the subject.[22,23]

Single-Agent Chemotherapy

Numerous single agents have been tested for activity against mesothelioma. In the past, many small studies reported relative success, but these may have represented a bias toward publishing positive results. Such favorable outcomes have usually not been confirmed by larger, follow-up series. Table 4 summarizes the larger single-agent chemotherapy trials that have included ≥15 patients.[12-14,17-19,22,24-59] Smaller trials have not been included in Table 4, but some are mentioned in the text.

Anthracyclines/DNA Intercalating Agents

Historically, the anthracyclines have been considered to be active against mesothelioma, although larger and more recent trials have shown no more than modest efficacy among these agents. Doxorubicin, the most commonly cited anthracycline, has been used in a number of studies over the last 2 decades. This drug has demonstrated some definite activity against mesothelioma, with overall response rates of 10 to 20%.[23] In larger trials, the results have been variable. Sorensen et al[24] reported no responses among 15 patients who were given doxorubicin at a dose of 60 mg/m^2, whereas Lerner et al[25] noted a 14% response rate among 51 patients given the same dose. Most studies of doxorubicin have used doses <75 mg/m^2 given every 3 weeks.[22,23] Higher-dose doxorubicin trials may be warranted, as several reports of combination chemotherapy[60,61] have suggested increased efficacy with dose-escalated doxorubicin.

Other tested anthracyclines include detorubicin, epirubicin, and pirarubicin. Among these agents, detorubicin at a dose of 40 mg/m^2 achieved a 26% response rate in a trial of 35 patients,[26] whereas pirarubicin given at 70 mg/m^2 produced response rates of 9% among 35 patients[27] and 33% in 15 patients.[28] These favorable outcomes need confirmation in further studies. The other agents in this class have shown minimal activity with the exception of high-dose epirubicin (110 mg/m^2), which yielded seven partial responses (PRs) among 48 patients (15%).[29,30]

Unique DNA intercalating agents, including mitoxantrone,[31,32] menogaril,[33] amsacrine,[34] and diaziquone,[35] have shown no significant clinical activity against malignant mesothelioma.

Platinum Compounds

Cisplatin and carboplatin have been tested in a total of five large trials. Cisplatin, given every 4 to 6 weeks at a dose of 120 mg/m^2, produced a 13% response rate in one trial of 24 patients.[36] A Southwest Oncology Group study using 100 mg/m^2 reported a similar 14% response rate among 35 patients.[37] In a smaller trial, Planting et al[62] observed five responses among 14 patients (36%) with dose-intensive cisplatin (80 mg/m^2 weekly for 6 weeks), suggesting a possible advantage for high-dose therapy. Carboplatin has been tested in three larger studies, with responses ranging from 6 to 16%.[12,38,39]

Plant Alkaloids

The vinca alkaloids have not shown activity against mesothelioma. Vindesine produced only one response among 17 patients in a series using 3 mg/m^2/wk and no responses in a study of 2 mg/m^2/wk.[40,41] Single trials of vinblastine and vincristine have been equally disappointing, with no responses observed in either study.[42,43]

Poor results have also been seen with low-dose oral

Table 2—*Staging System for Malignant Mesothelioma, as Proposed by IMIC

	Definition
Tumor	
T1a	Ipsilateral parietal pleural involvement only
T1b	Ipsilateral parietal pleural involvement with scattered foci of visceral pleura involvement
T2	Involvement of parietal, mediastinal, diaphragmatic, and visceral pleura and at least one of the following: Diaphragmatic muscle involvement Confluent visceral pleural tumor Extension from visceral pleura into pulmonary parenchyma
T3	Involvement of parietal, mediastinal, diaphragmatic, and visceral pleura and at least one of the following: Endothoracic fascia involvement Extension into mediastinal fat Solitary focus of tumor extending into soft tissues of chest wall Nontransmural pericardial involvement
T4	Involvement of parietal, mediastinal, diaphragmatic, and visceral pleura and at least one of the following: Diffuse extension or multifocal masses of tumor in chest wall Direct transdiaphragmatic extension to peritoneum Direct extension to contralateral pleura Direct extension to mediastinal organs Direct extension to spine Extension to internal surface of pericardium, or myocardial involvement
Lymph nodes	
NX	Cannot assess
N0	No nodal metastases
N1	Ipsilateral bronchopulmonary or hilar node metastases
N2	Subcarinal or ipsilateral mediastinal node metastases, including ipsilateral mammary nodes
N3	Contralateral mediastinal, contralateral internal mammary, ipsilateral, or contralateral supraclavicular node metastases
Metastases	
MX	Cannot assess
M0	No distant metastases
M1	Distant metastases
Stage	
Ia	T1aN0M0
Ib	T1bN0M0
II	T2N0M0
III	Any T3M0 Any N1M0 Any N2M0
IV	Any T4 Any N3 Any M1

*IMIC=International Mesothelioma Interest Group. Modified from Rusch et al.[5]

Table 3—*Prognostic Groups in Malignant Mesothelioma*

Prognostic Group	Defining Variables	Median Survival, mo
1	PS=0, age <49 yr PS=0, age ≥49 yr, Hgb ≥14.6	13.9
2	PS=1 or 2, WBC <8.7, no chest pain	9.5
3	PS=0, age ≥49 yr, Hgb <14.6 PS=1 or 2, WBC <15.6, chest pain, no weight loss, Hgb ≥12.3 PS=1 or 2, 9.8 ≤WBC <15.6, chest pain, weight loss, Hgb ≥11.2	9.2
4	PS=1 or 2, 8.7 ≤WBC <15.6, no chest pain	6.5
5	PS=1 or 2, WBC <15.6, chest pain, no weight loss, Hgb <12.3 PS=1 or 2, 9.8 ≤WBC <15.6, chest pain, weight loss, Hgb <11.2 PS=1 or 2, WBC <9.8, chest pain, weight loss	4.4
6	PS=1 or 2, WBC ≥15.6	1.4

*PS=ECOG performance status; WBC=WBC count ($\times 10^9$/L); Hgb=hemoglobin (g/dL). Modified from Herndon et al.[20]

etoposide (100 mg/d), which yielded only one PR (4% response rate) in a trial of 23 patients.[44] Smaller studies using either oral or IV etoposide have not shown any responses.[23]

The CALGB tested paclitaxel at a dose of 250 mg/m^2, and only three disease regressions were observed among 35 patients (9%).[19] The European Organization for Research and Treatment of Cancer has recently reported a similarly low response rate for this agent.[63]

Alkylating Agents

Ifosfamide and cyclophosphamide have shown minor activity against mesothelioma in clinical trials. Cyclophosphamide, given at a dose of 1.5 g/m^2 every 3 weeks, produced no responses in a trial of 16 patients,[24] although reviews have quoted response rates of up to 26% with data pooled from small studies.[4]

Four larger trials using single-agent ifosfamide reported response rates ranging from 3 to 24%.[45-48] ECOG, using ifosfamide at a dose of 1.5 g/m^2 for 5 days of a 21-day cycle, reported only one PR among 40 patients (3%).[45] In a trial of ifosfamide (2 g/m^2 given for 4 days of a 21-day cycle),[46] SWOG reported two PRs among 26 patients (8%). Alberts et al[47] achieved the highest response rate with ifosfamide (1.2 g/m^2 or 1.5 g/m^2 given for 5 days of a 21-day cycle), reporting four PRs among 17 patients (24%). The same high response rate of 24% was reported by a Turkish group, in a trial in which 21 patients received ifosfamide, 1.2 or 2.3 g/m^2/d for 5 days in 3-week cycles.[48] There appeared to be a dose-response effect, with a 2.5-fold increased response rate observed in the higher-dose group, but at the cost of significant myelotoxic reactions. The composite response rate for these four ifosfamide trials is 12%.

Mitomycin, used at a dose of 10 mg/m^2 every 4 to 6 weeks, produced four PRs in 19 patients (21%), but with notable pulmonary toxic reactions.[49]

Other alkylating agents, including mechlorethamine,

Table 4—*Single-Agent Chemotherapy Trials (≥15 Patients) for Mesothelioma

Agent	No. of Studies	No. of Patients	Composite Response, %
DNA intercalators			
Doxorubicin	2[24,25]	66	11
Detorubicin	1[26]	35	26
Pirarubicin	2[27,28]	50	16
Epirubicin	2[29,30]	69	12
Mitoxantrone	2[31,32]	62	5
Menogaril	1[33]	22	5
Amsacrine	1[34]	19	5
Diaziquone	1[35]	20	0
Platinum compounds			
Cisplatin	2[36,37]	59	14
Carboplatin	3[12,38,39]	88	11
Plant alkaloids			
Paclitaxel	1[19]	35	9
Vindesine	2[40,41]	38	3
Vincristine	1[42]	23	0
Vinblastine	1[43]	20	0
Etoposide	1[44]	23	4
Alkylating agents			
Cyclophosphamide	1[24]	16	0
Ifosfamide	4[45-48]	104	12
Mitomycin	1[49]	19	21
Antimetabolites			
Acivicin	1[50]	19	0
Methotrexate	1[51]	60	37
Trimetrexate	1[14]	51	12
Edatrexate	1[17,18]	37	22
DHAC	1[13]	41	17
Dideazafolic acid (CB3717)	1[52]	18	6
Fluorouracil	1[53]	20	5
Biological response modifiers			
BCG	1[54]	30	0
Interferon-α-2a	1[55]	25	12
Interferon-γ†	1[56]	89	19
Interleukin-2†	2[57,58]	32	34
P30 protein	1[59]	15	13

*Adapted from Ong and Vogelzang.[22]
†Intrapleural administration.

thiotepa, and melphalan, have been tested in very small studies from which no conclusions can be accurately drawn.[4,23]

Antimetabolites

The antifolates have shown promise in the treatment of malignant mesothelioma. A favorable response rate of 37%, including one complete response (CR), was shown by Solheim et al[51] in a group of 60 patients who received high-dose methotrexate (3 g total dose at 10- and 21-day intervals).

Variable activity has been seen with two newer antifolates, trimetrexate and edatrexate, in trials by the CALGB. In the trimetrexate study, two dose regimens used sequentially (6 and 10 mg/m^2) yielded a 12% PR rate in both arms, with no CRs.[14] Patients receiving the higher dose demonstrated a longer median survival (8.9 months vs 5 months in the lower-dose group), suggesting a possible advantage for higher-dose therapy with this class of agents. Both dose groups, however, had the same 2-year survival rate (18%).

Edatrexate, given at a dose of 80 mg/m^2 to 20 patients, achieved a 25% response rate, including one CR.[17] Due to occasional reports of severe toxic reactions, the study continued using leucovorin with the same dose of edatrexate. Among the first 17 patients receiving this regimen, a response rate of 18% has been reported.[18] Forty patients have entered the trial, and results are due for analysis shortly.

A CALGB trial of dihydro-5-azacytidine (DHAC) given at a dose of 1.5 g/m^2 resulted in a 17% response rate, including one CR, with 70% of patients experiencing the drug's unusual toxic reaction of moderate-to-severe chest pain.[13] In a smaller trial of 14 patients, Dhingra et al[64] did not show any significant antitumor activity with this agent.

Dideazafolic acid, another antifolate, has been tested in a single study that reported a 6% response rate in 18 patients.[52] Among the other antimetabolites, fluorouracil (10 to 15 mg/kg) has been tested in one larger trial, producing a 5% response rate in 20 patients.[53] Small trials of DHAC[23] and a single trial with the amino acid analogue acivicin[50] have not yielded any responses.

Biological Response Modifiers

Various biological response modifiers and cytokines have been investigated for activity against mesothelioma in *in vitro* and *in vivo* studies and have been brought to clinical trials.[65] Agents tested in clinical trials include bacillus Calmette-Guérin (BCG), interferon-α, interferon-β, interferon-γ, and interleukin-2. BCG induced no responses,[54] while interferon-α-2a (3 mU/d) produced a 12% response in 25 patients.[55] Interferon-α has also been combined with other chemotherapeutic agents with mixed results, as described later in this article. Though Von Hoff and Huong[66] demonstrated *in vitro* suppression of growth of primary human mesothelioma cells by interferon-β, a clinical trial of this agent in 14 patients did not result in any responses and was fraught with side effects.[67] Interferon-γ administered interpleurally achieved an encouraging 45% response rate in patients with Butchart's stage I disease, but only a 6% response in those with stage II disease, yielding an overall response rate of 19%.[56] Of note, there were eight CRs among 89 patients, with a mean duration of response of 17 months. Intrapleural interleukin-2 has been evaluated in several trials, achieving favorable response rates of 24 to 47%.[57,58] These data warrant further investigation.

P30 protein (Onconase), a novel RNAase derived from frog eggs, has been studied in mesothelioma, and responses were seen in 2 of 15 patients.[59] Toxic reactions were confined to arthralgia and peripheral edema.

Combination Chemotherapy

Combination chemotherapy regimens have been evaluated in a number of studies, but have not shown any clear advantage over single-agent chemotherapy (Table 5).[11,15,16,22,61,68-88] Most combination chemotherapy

Table 5—*Combination Chemotherapy Trials (≥15 Patients) for Malignant Mesothelioma**

Combination	No. of Studies	No. of Patients	Composite Response, %
Doxorubicin based			
+Cyclophosphamide	1[68]	36	11
+Cyclophosphamide +DTIC	2[68,69]	60	17
+Ifosfamide	2[61,70]	38	24
+Cisplatin	2[11,71]	59	19
+Cisplatin +Mitomycin	1[72]	23	21
+Cisplatin +Cyclophosphamide	1[73]	23	26
+Cisplatin +Mitomycin +Bleomycin	1[74]	25	44
+5-azacytidine	1[75]	36	22
+Interferon-α	1[76]	25	16
Cisplatin based			
+Mitomycin	1[11]	35	26
+Vinblastine	1[77]	20	25
+Etoposide	2[78,79]	51	18
+Pirarubicin	1[80]	38	13
+DHAC	1[15,16]	30	13
+Interferon-α +Tamoxifen	2[81,82]	61	16
+Interferon-α +Mitomycin	2[83,84]	40	15
+Interferon-α	1[85]	23	35
Other			
Epirubicin/ifosfamide	1[86]	17	6
Zorubicin/DTIC	1[87]	23	0
Carboplatin/interferon-α	1[88]	15	7

*DTIC=dacarbazine. Adapted from Ong and Vogelzang.[22]

studies have included doxorubicin, cisplatin, or an alkylating agent, reflecting the popularity of these drugs based on their mild efficacy in single-agent trials.

Doxorubicin-Based Combinations

Doxorubicin has been combined with alkylating agents in more than five separate studies. Doxorubicin (60 mg/m^2) in combination with cyclophosphamide (500 mg/m^2) resulted in an 11% response rate among 36 patients; the addition of dacarbazine (250 mg/m^2) did not improve response in the other study arm of this trial (13% response rate among 40 patients).[68] However, an older study[69] reported a 25% response rate in 20 patients given these three agents at higher doses (doxorubicin, 60 to 90 mg/m^2; cyclophosphamide, 600 to 900 mg/m^2; and dacarbazine, 1 g/m^2). Carmichael et al[70] reported a 12.5% response rate among 16 patients given doxorubicin (40 mg/m^2) with ifosfamide (5 g/m^2). A smaller study of only 10 patients noted a 30% response rate for this combination when doxorubicin was given at a dose of 60 mg/m^2.[89] A phase II trial of dose-escalated doxorubicin (75 mg/m^2) plus ifosfamide (5 g/m^2) with growth factor support resulted in seven responses in 22 patients (32%); response duration, however, was only 6 months, and cumulative thrombocytopenia was dose limiting.[61] These studies suggest a possible dose-response effect for doxorubicin. The response rates to doxorubicin combined with an alkylating agent appear to be greater than those seen with single-agent doxorubicin, but the 95% confidence intervals for these regimens overlap, and median survivals seem to be similar in patients receiving either the single-agent or combination chemotherapy.

Doxorubicin has also been evaluated with cisplatin. In a study conducted by the CALGB,[11] doxorubicin (60 mg/m^2) was given with cisplatin (75 mg/m^2) every 4 weeks, resulting in five responses among 35 patients (14%). An Italian study reported a more favorable 25% response rate using a similar dosing regimen (both drugs given at a dose of 60 mg/m^2).[71] The same group has recently reported a 21% response rate with the addition of mitomycin (10 mg/m^2) to these two agents.[72] A comparable response rate of 26% was seen with doxorubicin (50 mg/m^2) and cisplatin (50 to 80 mg/m^2) when given with cyclophosphamide (500 mg/m^2).[73]

A four-drug trial of doxorubicin/cisplatin/mitomycin/bleomycin reported a response rate of 44%,[74] but this has not been confirmed. Doxorubicin in combination with 5-azacytidine (a compound similar to DHAC) yielded a 22% response in 36 patients,[75] whereas doxorubicin combined with interferon-α induced only a 16% response rate in 25 patients.[76]

Cisplatin-Based Combinations

Among the cisplatin-based regimens, cisplatin given with mitomycin in one arm of CALGB 8435 resulted in a 26% response rate, including two CRs.[11] Tsavaris et al[77] evaluated cisplatin (100 mg/m^2) combined with vinblastine (6 mg/m^2) in 20 patients, reporting one CR and four PRs (25% response) with a 13-month mean duration of response. One study in which cisplatin (70 mg/m^2/wk for 6 of 7 weeks) was administered with oral etoposide (50 mg/d) to 25 patients resulted in one CR and five PRs (24% response),[78] whereas another study reported a 12% response rate with this combination.[79] Cisplatin has also been tested in combination with DHAC[15,16] and with pirarubicin,[80] but no improvements over single-agent cisplatin therapy were reported.

Other Combinations

The anthracyclines epirubicin and zorubicin, given in combination with ifosfamide[86] and dacarbazine,[87] respectively, have shown poor responses.

Immunotherapeutic and chemotherapeutic agents have been combined in several clinical trials. Interferon-α (5 mU/m^2) combined with tamoxifen (20 mg orally twice a day) and cisplatin (25 mg/m^2) was used in a series of 25 patients, yielding three PRs (12%).[81] In a slightly larger study by the National Cancer Institute,[82] this same combination produced seven PRs among 36 patients (19%) with a mean duration of response of only 6 months. Another trial of 25 patients showed a response rate of 16% when daily interferon-α was combined with weekly doxorubicin for 12 weeks.[76] Interferon-α-2b, given in combi-

nation with cisplatin and mitomycin, yielded 11% and 19% response rates in two separate studies.[83,84] Although the addition of interferon-α to these chemotherapeutic regimens did not improve response rates, another study combining interferon with weekly cisplatin (60 mg/m^2) reported a 35% response rate in 23 patients, with a median survival of 25 months for responders vs 8 months for nonresponders.[85] The authors of this study admitted that the improved response rate could have been due to the dose-escalated cisplatin that was used. In preliminary results, higher-dose interferon-α (24 mU/wk) combined with cisplatin has not shown an advantage over the lower dose, with the former producing 27% response among 15 patients.[90] Finally, interferon-α-2a and carboplatin induced only one response in 15 patients (7% response rate).[86]

Conclusion

Despite many investigations, no chemotherapeutic protocol has yet emerged as an effective treatment for malignant pleural mesothelioma. Previous studies have been hindered by small size, inability to accurately measure disease extent, absence of a precise staging system, and lack of knowledge regarding the natural history of the disease. Cohesive, multi-institutional studies have become necessary to approach this disease in an efficient manner.

Among the chemotherapy agents that have been tested, the anthracyclines, platinum compounds, and alkylating agents have demonstrated small but real activity against mesothelioma. Preliminary evidence suggests an advantage to dose-escalated administration of doxorubicin and cisplatin, indicating that mesothelioma may not be totally chemotherapy-resistant, and further investigations with such dosing regimens may be warranted. The antifolates have shown increased activity against this disease and should be studied further. Also intriguing are the favorable results seen with intrapleural administration of interferon-γ and interleukin-2 and novel systemic agents such as P30 protein. Combination chemotherapy regimens, however, have not shown any clear benefit over single-agent therapy.

Randomized trials are needed to confirm the activity shown by agents in phase II studies. New agents need to be tested for activity against mesothelioma. Agents that warrant investigation include the topoisomerase I inhibitors and newer antimetabolites, such as gemcitabine. By adopting the new TNM staging system and utilizing new knowledge regarding prognostic indicators, we can hopefully construct more precise investigations into the treatment of malignant mesothelioma, and thereby better interpret future results.

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Phase I Study of an Oral Histone Deacetylase Inhibitor, Suberoylanilide Hydroxamic Acid, in Patients With Advanced Cancer

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Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

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ABSTRACT

Purpose
To determine the safety, dosing schedules, pharmacokinetic profile, and biologic effect of orally administered histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) in patients with advanced cancer.

Patients and Methods
Patients with solid and hematologic malignancies were treated with oral SAHA administered once or twice a day on a continuous basis or twice daily for 3 consecutive days per week. Pharmacokinetic profile and bioavailibity of oral SAHA were determined. Western blots and enzyme-linked immunosorbent assays of histones isolated from peripheral-blood mononuclear cells (PBMNCs) pre and post-therapy were performed to evaluate target inhibition.

Results
Seventy-three patients were treated with oral SAHA and major dose-limiting toxicities were anorexia, dehydration, diarrhea, and fatigue. The maximum tolerated dose was 400 mg qd and 200 mg bid for continuous daily dosing and 300 mg bid for 3 consecutive days per week dosing. Oral SAHA had linear pharmacokinetics from 200 to 600 mg, with an apparent half-life ranging from 91 to 127 minutes and 43% oral bioavailability. Histones isolated from PBMNCs showed consistent accumulation of acetylated histones post-therapy, and enzyme-linked immunosorbent assay demonstrated a trend towards a dose-dependent accumulation of acetylated histones from 200 to 600 mg of oral SAHA. There was one complete response, three partial responses, two unconfirmed partial responses, and 22 (30%) patients remained on study for 4 to 37+ months.

Conclusions
Oral SAHA has linear pharmacokinetics and good bioavailability, inhibits histone deacetylase activity in PBMNCs, can be safely administered chronically, and has a broad range of antitumor activity.

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INTRODUCTION

Histone deacetylases (HDACs) are enzymes that regulate chromatin structure and function through the catalysis of the removal of the acetyl modification from lysine residues of histones.[1] While the base sequence of DNA provides the fundamental code for proteins, post-translational modification of proteins plays a major role in the control of gene transcription. The amino acid tails of the core nucleosomal histones are subject to post-translational modifications by acetylation of lysines, methylation of lysines and arginines, phosphorylation of serines, and ubiquitination of lysines. The most extensively studied post-translational modification of histones is the acetylation of

lysine. The opposing activities of HDACs and histone acetyl transferases (HATs) regulate the balance of acetylation of histones. HDACs are also involved in reversible acetylation of nonhistone proteins, such as p53, tubulin, various transcription factors, and other proteins.[2-4]

HDAC inhibitors have been shown to cause cultured, transformed cells to undergo growth arrest, terminal differentiation, apoptosis, or autophagic cell death.[1] These agents act selectively in altering the transcription of relatively few of the expressed genes (generally 2% to 10% of expressed genes are increased or decreased in their rate of transcription).[5-8] HDAC inhibitors have been found to be additive and even synergistic with a number of anticancer agents including radiation, anthracyclines, flavopiridol, imatinib, proteasome inhibitors, and all *trans*-retinoic acid in blocking the proliferation or inducing apoptosis in tumor cells in culture.[1]

Inhibitors of HDAC represent a new class of targeted anticancer agents. A number of structural classes of HDAC inhibitors have been developed and are in clinical trials, including short chain fatty acids (the benzamides [MS-275]), the cyclic peptide, depsipeptide (FK-228), and suberoylanilide hydroxamic acid (SAHA).[1] We have previously reported the results of a phase I clinical trial of the HDAC inhibitor, SAHA, administered intravenously (IV) to patients with solid or hematologic malignancy.[9] This trial showed that SAHA caused an accumulation of acetylated histones in normal and malignant cells post-therapy, was well tolerated, and had antitumor activity, indicating the agent was effective in reaching and inhibiting its targets. These data, supported by in vitro studies, suggested that daily administration of SAHA may improve the therapeutic benefit, and an oral formulation was developed to improve the feasibility of daily administration. We now report the results of the phase I clinical study with orally administered SAHA in patients with advanced cancers.

Patients and Methods

Patient Eligibility

Adult patients with solid tumors or hematologic malignancies who had failed or relapsed from standard therapy were eligible. All patients were required to have a Karnofsky performance status $\geq$ 70% and adequate hepatic and renal function. Solid tumor patients were required to have platelet count $\geq$ 100,000 cells/μL and WBC $\geq$ 3,500 cells/μL, and patients with hematologic malignancies were required to have an absolute neutrophil count $\geq$ 500 cells/μL and a platelet count $\geq$ 25,000 cells/μL. All patients with solid tumors were required to have radiographic evidence of measurable or nonmeasurable metastatic disease. Patients were required to have recovered from the acute toxicities of any prior therapy, and no chemotherapy, radiation therapy, or other investigational anticancer therapy for a minimum of 4 weeks before initiating the protocol. Leukemia patients could have received conventional cytotoxic therapy and lymphoma patients could receive steroids up to 2 weeks before starting therapy.

Patients with clinically significant cardiac or pulmonary disease, active CNS disease, or active infection were not eligible. Pregnant women and lactating females were excluded. The study was approved by the Memorial Sloan-Kettering Cancer Center (New York, NY) institutional review board, and all patients signed an informed consent.

Trial Design and Treatment Plan

Oral SAHA was provided by Aton Pharma, Inc (Tarrytown, NY) as 200-mg capsules and later in the study a 50-mg capsule was available. The gelatin capsules contain SAHA, and standard pharmaceutical excipients (microcrystalline cellulose, sodium croscarmellose, and magnesium stearate). The starting dose was one tenth the maximum tolerated dose (MTD) in nonrodent species (90 mg/m^2/d or approximately 200 mg daily). Due to the initial availability of only the 200-mg capsule, and preclinical data predicting poor bioavailability of SAHA, a fixed dosing schedule was used. There were eight cohorts of oral SAHA studied. SAHA was given daily at 200 mg qd, 400 mg qd, 600 mg qd, or 400 mg bid (cohorts 1 to 4). The dose was planned to be escalated to 1,200 mg bid, however, dose-limiting toxicity (DLT) was encountered at 400 mg bid. The protocol was amended to evaluate 200 mg bid and 300 mg bid dose levels (cohorts 5 and 6) and an intermittent dosing schedule of 300 mg bid and 400 mg bid for three consecutive days weekly (cohorts 7 and 8). Dose escalation proceeded independently in patients with solid tumors and hematologic malignancies. To minimize exposing patients to subtherapeutic treatment, hematologic patients were enrolled in cohorts 2 to 5. Patients were instructed to take oral SAHA at home in a fasting state, but later were allowed to take with food. Patients recorded the date and time of the ingestion of the oral SAHA capsule(s), and a pill count was performed to evaluate compliance and accountability of the study drug.

A treatment cycle was 4 weeks of therapy. DLT was defined as: grade 4 neutropenia or thrombocytopenia; grade 3 neutropenia with fever (solid tumor patients only); and grade 3 or 4 nonhematologic toxicity (solid tumor and hematologic malignancy patients) during the first cycle of therapy. A treatment delay due to toxicity that lasted longer than 1 week was also considered a DLT. At least three patients were entered per cohort and individual cohorts were expanded to six patients after the development of one DLT. MTD was defined as the highest dose with an observed incidence of DLT in no more than one of six patients treated at a dose level. At least six and as many as 20 patients would be treated at the MTD in the solid tumor and hematologic malignancy groups. Toxicities were evaluated by the National Cancer Institute Common Toxicity Criteria (version 2.0).

Patient Evaluation

The pretreatment evaluation included history and physical examination (H&P), Karnofsky performance status, a complete CBC, hepatic and renal function tests, coagulation profile (prothrombin time/partial thromboplastin time), urinalysis, and chest x-ray. A pregnancy test was obtained in women with childbearing potential. Appropriate tumor markers were obtained in patients with prostate or breast cancer. Imaging studies included a chest, abdominal, and pelvic computed tomography (CT) scan, magnetic resonance imaging scan, and positron emission tomography or bone scan as clinically indicated. All patients had a baseline ECG and further cardiac work-up if indicated.

Patients were evaluated weekly with H&P and laboratory tests (CBC, hepatic\renal function, prothrombin time/partial thromboplastin time) and urinalysis during the first 8 weeks of therapy. Tumor markers were repeated every 2 weeks and imaging studies every 8 weeks. An ECG was obtained before every cycle of therapy. If the patient was on study longer than 8 weeks, H&P and laboratory tests were performed every other week for 8 weeks and then monthly thereafter with imaging studies performed every 4 months.

All patients were assessed for toxicity and response if they received any treatment. In patients with measurable disease, standard WHO phase II response criteria[10] were utilized and radiographs underwent a blinded review by a radiologist. The Cheson criteria were employed for patients with lymphoma and leukemia.[11,12]

Pharmacokinetics Studies. Pharmacokinetic (PK) studies were performed during the first cycle of therapy in 44 patients. To assess the absolute bioavailability of oral SAHA, the initial 18 patients received a 2-hour infusion of intravenous SAHA that was equivalent to the assigned oral dose of SAHA on day 1 of therapy. Blood (10 mL) was drawn at time 0, 30, 60, 115, 135, 150, 180, 210, 240, 300, and 360 minutes following the intravenous infusion of SAHA. After 1 week without receiving SAHA, patients started the oral SAHA (day 8). On day 8 (fasting), patients fasted (no food or beverage other than water for 2 hours before the ingestion of SAHA) before the administration of oral SAHA, and on day 9 (fed), all patients received a standardized meal (a bagel with cream cheese or butter, a pint of orange juice, and a cup of coffee with milk and sugar) 30 minutes before the ingestion of SAHA capsule(s). PK studies were performed on days 8 and 9 and on day 15 or 22. Four patients that were on the oral SAHA therapy for more than 6 months repeated the PK study. PK study consisted of drawing 10 mL of heparinized blood at time 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, and 600 minutes following oral SAHA ingestion.

Blood samples were placed on ice and refrigerated until they were centrifuged to separate the plasma. The plasma (3 to 5 mL) was transferred to a labeled conical 15 mL polypropylene screw top tube and stored at −20°C. Determination of SAHA concentrations in plasma samples was conducted in the Pharmacology Analytical Laboratory at the Memorial Sloan-Kettering Cancer Center. A liquid chromatography-mass spectrometry method was employed. A 450-μL aliquot of thawed plasma was mixed with 50 μL of d_5-SAHA (internal standard). The plasma was filtered through 0.45-μm cellulose acetate filters (Costar, Corning, NY) by centrifugation at 3,000 × g for 12 minutes. A 200-μL aliquot of filtrate was transferred to an autosampler vial. An injection volume of 30 μl was directly injected through a Prospekt 2 in-line solid phase extraction system (Spark-Holland, Emmen, the Netherlands), which consisted of a high-pressure eluter and an automated cartridge exchanger. After washing the cartridge with water, the mobile phase was changed to methanol: 0.1%:formic acid (1:1, vol:vol) at a flow rate of 0.4 mL/min, and passed over to a Reliance SBC8 4 mm × 80 mm column (Agilent Technologies, Wilmington, DE). The eluant was assayed using a diode array UV/VIS detector (at 240 nm), and an Agilent mass spectrometer detector (Agilent Technologies). The mass spectrometer detector was operated in the atmospheric pressure chemical ionization–positive mode. The masses monitored were 265 for SAHA and 270 for d_5-SAHA. The detection limit for SAHA was about 15 ng/mL, and the response was linear from 15 ng/mL to 1,000 ng/mL ($r^2 > 0.99$).

The area under the plasma concentration time curve (AUC) was calculated using the linear trapezoid method. The terminal elimination rate constant, λz, was calculated as the negative of the slope of the terminal log-linear portion of the plasma concentration time curve. Total plasma clearance (CL) and volume of distribution (Vz) were calculated using standard formulas without correcting for bioavailability. The bioavailability after oral administration (F) was calculated for each patient at a given dose on the day of sampling ($F = AUC_{oral}/AUC_{IV} \times Dose_{oral}/Dose_{IV}$). All PK calculations were performed using noncompartmental methods with WinNonLin version 3.1 (Pharsight Corp, Mountain View, CA).

Correlative Studies. Effect of SAHA on histone acetylation in mononuclear cells was assessed by Western blotting and enzyme-linked immunosorbent assay (ELISA). Peripheral blood (10 to 30 mL) was obtained in heparinized tubes at pretreatment, 2 hours postinfusion and between 2 to 10 hours after ingestion of SAHA capsule(s). Histones were isolated from the peripheral-blood mononuclear-cells (PBMNCs) and acetylated histone H3 was evaluated by Western blot analysis as previously described.[9]

For the ELISA, 50 to 100 ng of histone extract was passively adsorbed to triplicate wells of Immulon microtiter plates (VWR, West Chester, PA) and incubated overnight at 4°C. After washing with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-Tween), the plates were blocked for 1 hour at room temperature using PBS-Tween containing 5% nonfat milk and 1% goat serum. Total and acetylated histone H3 levels were then quantified by using an antihistone H3 rabbit polyclonal (Abcam, Cambridge, MA) or an antiacetylated histone H3 rabbit polyclonal (Upstate Biotechnology, Lake Placid, NY), respectively. The primary antibodies were diluted in PBS-Tween containing goat serum (0.5%) and added to the appropriate microtiter plates. After 1 hour at room temperature, the plates were washed with PBS-Tween and a horseradish peroxidase–conjugated goat antirabbit secondary antibody (Bio-Rad, Hercules, CA) was added. The plates were incubated at room temperature for 1 hour and then extensively washed with PBS-Tween. Horseradish peroxidase signals were visualized using the TMB peroxidase substrate kit (Bio-Rad) according to manufacturer's instructions. Normalized histone H3 acetylation levels for each sample were derived by dividing the acetylated histone H3 optical density value by the total histone H3 optical density value derived independently from the same sample. All samples within each patient set were normalized to the level of histone H3 acetylation from the pretreatment sample of that patient, which was assigned a value of 1.

RESULTS

Patient Characteristics and Treatment Administration

Seventy-six patients were enrolled onto the study and 73 received at least one dose of oral SAHA. Three patients did not receive the study drug due to the development of brain metastasis in one and rapid disease progression in two patients. The most common tumors were mesothelioma (n = 13) and non-Hodgkin's lymphoma (n = 12). Patient characteristics are described in Table 1. Seventy-eight percent of all patients had received two or more prior systemic therapies. The majority of hematologic

Table 1. Patient Characteristics (N = 73)

Characteristic	Solid Tumors (n = 50)	Hematologic Malignancies (n = 23)
Age, years		
Median	60	59
Range	25-78	20-79
Sex		
Male	34	16
Female	16	7
Primary tumor type		
Mesothelioma	13	
Prostate	7	
Urothelial	7	
Thyroid	6	
Renal	6	
Breast	2	
Lung	2	
Adrenal cortical	1	
Germ cell	1	
Laryngeal	1	
Melanoma	1	
Paraganglioma	1	
Skin	1	
Cervical	1	
Hodgkin's lymphoma		7
Non-Hodgkin's lymphoma		
Diffuse large B-cell		7
Small lymphocytic		1
Mantle cell		2
Cutaneous T-cell		1
Peripheral T-cell		1
Myeloma		2
Acute myeloid leukemia		1
Myelodysplastic syndrome		1
No. of prior systemic therapies: chemotherapy, or biologic therapy, or both		
None	1	0
One	14	1
Two	11	4
Three or more	24	18

malignancy patients had received three or more prior systemic therapies (n = 18; 78%). Seventy-three patients received a total of 416 treatment cycles. The median number of treatment cycles was two (range, one to 37+ cycles), which was the same in patients with solid tumor or hematologic malignancies. Twenty-two patients (solid tumor, n = 16; hematologic malignancy, n = 6) completed four or more treatment cycles. Eight completed 12 or more treatment cycles and four patients are still on study with the longest treatment duration exceeding 37 cycles in two patients.

Fifty-six patients (77%) were discontinued from the study because of progressive disease (solid tumor, n = 40; hematologic malignancy, n = 16). Ten patients (14%) were discontinued because of adverse event (solid tumor n = 7; hematologic malignancy n = 3), including one patient with widely metastatic mesothelioma who died of infection without neutropenia during the second week of treatment. The death was considered unlikely to be caused by the study drug. Three patients with hematologic malignancies were removed from the study for protocol violation, noncompliance, and patient withdrawal of consent.

DLT and MTD

The number of patients and DLT for each dose level for the solid tumor and hematologic patients are listed in Table 2. Most of the patients (n = 67, 92%) were treated at the MTD or above. The DLTs were predominantly anorexia, dehydration, diarrhea, and fatigue. The MTD for continuous daily dosing for hematologic and solid tumors was 400 mg qd or 200 mg bid, and for solid tumors 300 mg bid × 3 consecutive days per week. The dosing schedule did not appear to have a major effect on the pattern of DLTs and at the doses that exceeded the MTD; the frequency of DLT increased but the pattern and severity remained the same. At the 400 mg bid dose level, six of nine patients (hematologic and solid tumors) developed DLT in the first cycle of therapy and four of six DLTs occurred in 14 days or less after starting the oral SAHA. The median time to resolution of the DLTs was 7 days (range, 3 to 10 days).

Safety and Tolerability

The most common drug-related adverse events (Table 3) were constitutional (fatigue), gastrointestinal (anorexia, nausea, diarrhea, and vomiting), metabolic (hyperglycemia, and hypocalcemia), and hematologic (anemia and thrombocytopenia). Grade 4 events occurred in nine patients (solid tumor, three patients; hematologic malignancy, six patients), most of which were hematologic: anemia (n = 4), neutropenia (n = 1), thrombocytopenia (n = 1), hyponatremia (n = 1), elevation in creatine phosphokinase (CPK) (n = 1), and infection (n = 1). Overall, a higher incidence of grade 3 or 4 thrombocytopenia (21% *v* 36%) and grade 3 dehydration (21% *v* 36%) was observed in the twice-a-day continuous dosing schedule, more so in hematologic patients than in solid tumor patients. There were no grade 3 or 4 hematologic toxicities seen in the twice-a-day × 3 days-per-week–treated solid tumor patients, but an increase in the incidence of severe fatigue was noticed when compared with continuous twice daily and the once daily regimens (37% *v* 28% *v* 17%, respectively). More patients with hematologic malignancies experienced thrombocytopenia (87%) than solid tumor patients (44%), and thrombocytopenia was more severe in those patients. Grade 3 thrombocytopenia occurred in six solid tumor patients (12%) and eight patients with hematologic malignancies (35%), most of which (79%) resolved to grade 2 within 7 days. Bone marrow biopsies in two patients with grade 3 or 4 thrombocytopenia suggested that the most probable cause of thrombocytopenia was maturation arrest. Grade 3 neutropenia occurred in three hematologic patients and grade 4 in one, all of which resolved to grade 2 or less without intervention. No patients had neutropenic fever or discontinued therapy because of neutropenia. Fatigue was more common at the higher dose levels on the twice-a-day dosing regimens but was reversible within 3 to 7 days. Mild to moderate GI

Table 2. DLT and Dose Escalation

Dose Level	Dosing Regimen	Solid Tumor (N = 50)		Hematologic Malignancy (N = 23)	
		No. of Patients	DLT	No. of Patients	DLT
1	200 mg qd	6*	None	—	—
2	400 mg qd	5†	None	11‡	Dehydration/diarrhea (n = 1) Dehydration/diarrhea/fatigue (n = 1)
3	400 mg bid	6	Dehydration/diarrhea (n = 1) Fatigue (n = 1) Thrombocytopenia (n = 1)	3	Anorexia (n = 1) Dehydration (n = 1) Anorexia/dehydration (n = 1)
4	600 mg qd	4	Anorexia/dehydration/fatigue/nausea (n = 1)	3	Dehydration/diarrhea (n = 1) Diarrhea (n = 1)
5	200 mg bid	4	None	6	Anorexia (n = 1)
6	300 mg bid	6	Elevated ALT/AST (n = 1) Anorexia/fatigue (n = 1) Fatigue (n = 1)	—	—
7	300 mg bid × 3 days/week	13§	None	—	—
8	400 mg bid × 3 days/week	6	Fatigue (n = 2) Dehydration/nausea/vomiting (n = 1)	—	—

Abbreviations: DLT, dose-limiting toxicity; qd, every day, bid, twice a day.
*Three patients removed for early progression of disease (POD) during first 4 weeks.
†One patient removed for early POD, and one additional patient treated at this dose level.
‡No DLTs observed in the first five patients enrolled; six additional patients enrolled at the maximum tolerated dose (MTD), and two developed DLTs.
§Ten additional patients treated at the MTD.

symptoms of anorexia, diarrhea, nausea, and vomiting were common and antiemetics and antidiarrheal medications were able to control symptoms. Anorexia was associated with gustatory changes in 8% of the patients.

Twenty-five patients (34%) reported mild to moderate dyspnea without other associated cardiopulmonary symptoms or new abnormalities on the chest x-ray or ECGs. Serial ECGs showed nonspecific ST and QT changes but no consistent patterns were identified. No patients were found to have cardiac arrhythmias, new onset angina, or other cardiac toxicities.

Pharmacokinetics

PK parameters and mean plasma concentration time curves from patients treated with 200, 400, and 600 mg of SAHA are presented in Table 4 and Figure 1. The pharmacokinetics of SAHA after oral administration of a single dose of SAHA are linear from 200 to 600 mg (Fig 2). The mean apparent half-life ($t_{1/2}$) following oral administration (range, 91.6 to 127 minutes) was longer than the mean apparent $t_{1/2}$ following intravenous administration of the oral equivalent doses (range, 34.7 to 42.4 minutes). The estimated bioavailability of SAHA at doses of 200 and 400 mg administered during the fasting state was 43%. Exploratory studies in the fasting and nonfasting (fed) state suggest that oral administration of SAHA with food does not appear to substantially alter the rate or extent of absorption. PK parameters obtained in four patients after 6 months or more of therapy were similar to baseline values.

Table 3. Ten Most Common Drug-Related Toxicities in 73 Patients (all cycles, highest grade per event per patient)

	Solid Tumors (n = 50)		Hematologic Malignancy (n = 23)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Hematologic				
Anemia	36	5	10	7
Thrombocytopenia	16	6	11	9
Nonhematologic				
Anorexia	26	4	10	4
Diarrhea	19	2	12	7
Elevated serum creatinine	28	1	19	0
Fatigue	32	14	16	5
Hyperglycemia	37	6	17	3
Hypocalcemia	20	2	12	1
Nausea	36	3	15	0
Vomiting	23	2	7	1

Correlative Studies

Histone acetylation was evaluated by Western blot or ELISA on histones isolated from PBMNCs. Histone samples were isolated from PBMNCs from 50 patients, and accumulation of acetylated histone H3 (AcH3) was observed at 2 hours after oral ingestion of SAHA consistently in all dose cohorts (Fig 3). As the dose of oral SAHA was increased from 200 to 600 mg, the duration that an accumulation of AcH3 was observed increased from 4 to 10 hours. An accumulation of AcH3 in PBMNCs was consistently observed at all dose levels after 3 weeks of oral SAHA. Two patients that remained on study > 6 months had repeat analysis of the AcH3 (Fig 4). An increase in accumulation of AcH3 was observed in patients on prolonged treatment with oral SAHA.

Antitumor Activity

Twenty-two patients (30%) remained on study for 4 to 37+ months (Table 5). Of these 22 patients, there was one

Table 4. Pharmacokinetic Parameter for SAHA After Oral Administration

Day	Parameter*	200 mg qd	200 mg bid	300 mg bid	400 mg qd	400 mg bid	600 mg qd
1 (intravenous)	No. of patients	6	NA	NA	8	6	NA
	C_{max}, ng/mL	1,088 ± 567			2,306 ± 1,099	2,184 ± 1,253	
	T_{max}, min	60			60	60	
	AUC_{0-t}, min-ng/mL	105,300 ± 64,224			214,132 ± 133,211	201,020 ± 105,654	
	AUC_{∞}, min-ng/mL	124,518 ± 69,943			169,202 ± 27,794	210,588 ± 107,344	
	$T_{1/2}$, min	34.7 ± 13.4			42.4 ± 15.5	38.4 ± 9.2	
	CL/F, mL/min	2,134 ± 1,283			2,513 ± 439	2,231 ± 1,153	
	Vz/F, L	89.2 ± 23.1			150 ± 51.5	117 ± 51.8	
8 (fasted)	No. of patients	6	10	6	10	6	6
	C_{max}, ng/mL	304 ± 150	301 ± 152	263 ± 100	658 ± 439	349 ± 127	804 ± 397
	T_{max}, min	135	120	53	106	150	90
	AUC_{0-t}, min-ng/mL	43,426 ± 28,400	49,466 ± 38,766	41,489 ± 22,099	101,854 ± 105,570	66,439 ± 15,087	166,555 ± 86,736
	AUC_{∞}, min-ng/mL	40,393 ± 23,046	74,374 ± 74,914	39,730 ± 23,694	161,443 ± 169,849	77,334	139,370 ± 71,002
	$T_{1/2}$, min	91.6 ± 27.2	122 ± 33.8	93.5 ± 25.2	88.9 ± 20.5	100	127 ± 64.2
	CL/F, mL/min	5,987 ± 2,790	4,699 ± 2,994	11,006 ± 7,994	4,409 ± 2,682	5,172	5,418 ± 3,367
	Vz/F, L	853 ± 607	834 ± 617	1,528 ± 1,147	531 ± 346	748	889 ± 444
9 (fed)	No. of patients	6	10	6	9	6	6
	C_{max}, ng/mL	279 ± 151	295 ± 160	297 ± 111	667 ± 696	455 ± 158	685 ± 277
	T_{max}, min	120	150	105	90	105	150
	AUC_{0-t}, min-ng/mL	41,686 ± 24,516	52,074 ± 34,025	51,572 ± 18,183	134,292 ± 181,574	99,293 ± 60,407	174,322 ± 72,414
	AUC_{∞}, min-ng/mL	56,001 ± 37,054	48,120 ± 17,518	48,868 ± 21,839	199,874 ± 252,665	121,970 ± 99,787	236,094 ± 150,007
	$T_{1/2}$, min	70.5 ± 12.9	91.9 ± 39.3	97.8 ± 38.2	135 ± 109	100 ± 34.4	111 ± 58.2
	CL/F, mL/min	5,776 ± 5,256	4,518 ± 1,243	7,410 ± 4,069	3,945 ± 2,086	5,096 ± 3,657	3,184 ± 2,023
	Vz/F, L	522 ± 373	621 ± 359	1,016 ± 476	885 ± 1,139	616 ± 265	424 ± 56
22-30	No. of patients	3	7	5	7	2	3
	C_{max}, ng/mL	233 ± 88.8	263 ± 76.3	263 ± 89.9	446 ± 105	268 ± 78.4	334 ± 160
	T_{max}, min	45	45	60	90	195	120
	AUC_{0-t}, min-ng/mL	33,333 ± 23,678	39,634 ± 13,170	32,658 ± 9,714	65,324 ± 19,435	65,740 ± 29,677	49,602 ± 1,945
	AUC_{∞}, min-ng/mL	†	43,511 ± 14,009	30,759 ± 8,942	92,625 ± 8,461	88,106	75,489
	$T_{1/2}$, min	†	78 ± 46.9	57.6 ± 44.8	98 ± 31.3	43.4	685
	CL/F, mL/min	†	5,129 ± 2,149	10,400 ± 3,008	4,337 ± 396	4,540	7,948
	Vz/F, L	†	513 ± 207	963 ± 1,016	604 ± 140	284	7,851
> 6 months	No. of patients	NA	1	NA	NA	31	NA
	C_{max}, ng/mL		247			358 ± 67	
	T_{max}, min		127			80	
	AUC_{0-t}, min-ng/mL		33,949			47,883 ± 9,185	
	AUC_{∞}, min-ng/mL		34,640			47,904 ± 9,175	
	$T_{1/2}$, min		76			79 ± 30	
	CL/F, mL/min		5,890			8,540 ± 1,520	
	Vz/F, L		650			1,000 ± 450	

Abbreviations: SAHA, suberoylanilide hydroxamic acid; qd, every day; bid, twice a day; NA, not applicable; C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC, area under the curve; $T_{1/2}$, half-life; CL/F, clearance; Vz/F, volume of distribution.

*Mean ± SD except for T_{max}, for which the median is reported. Individual patients are reported if $n \leq 2$.

†Parameter could not be calculated

‡At the time of pharmacokinetic studies, one patient was on 400 mg bid and two patients were dose reduced to 400 mg daily.

complete response (CR) in a patient with transformed diffuse large B-cell lymphoma with normalization of the positron emission tomography scan and resolution of the bone marrow involvement for 17 months, and three partial responses (PRs) were noted in the following patients: de novo diffuse large B-cell lymphoma, laryngeal cancer (n = 1), and papillary thyroid cancer (n = 1). Two unconfirmed partial responses were observed in patients with metastatic mesothelioma. Stable disease was seen at all dose levels but confirmed CRs and PRs were only seen at 400 mg bid and 600 mg daily dose levels. An improvement in tumor-related pain and dyspnea was observed in patients with laryngeal cancer and mesothelioma who had tumor regression. Prolonged disease stabilization was also seen in patients with renal cell carcinoma and thyroid cancer with minor objective tumor regression. There were six patients with metastatic thyroid cancer (four poorly differentiated papillary, one Hürthle cell, and one medullary) maintained on oral SAHA for a median of 27 months (range, 12 to 37+ months). Three papillary thyroid patients had radioactive iodine (RAI) scans performed post-therapy and one had an improvement in the RAI scan post-therapy with oral SAHA.

DISCUSSION

This study demonstrates that oral SAHA can be administered safely for prolonged durations at doses that inhibit HDAC activity, has linear pharmacokinetics with good bioavailability, and has a broad range of antitumor activity. This study defined a once daily (400 mg qd), twice daily (200 mg bid), and a twice daily for 3 consecutive days every week (300 mg bid) dosing schedule that could be used safely in future studies. Fatigue, anorexia, dehydration, and diarrhea were the DLTs observed across the three dosing schedules. The DLTs and the doses at which DLTs occurred were similar between patients with hematologic malignancies

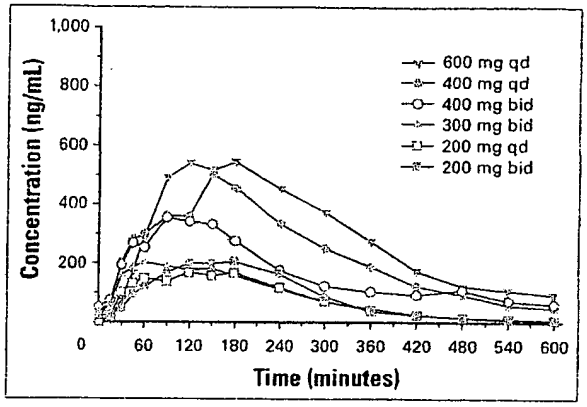

Fig 1. Mean plasma concentrations of suberoylanilide hydroxamic acid on cycle 1/day 9 after oral administration of 200 mg every day (qd) or twice a day (bid), 300 mg bid, 400 mg qd or bid, or 600 mg qd under fed conditions.

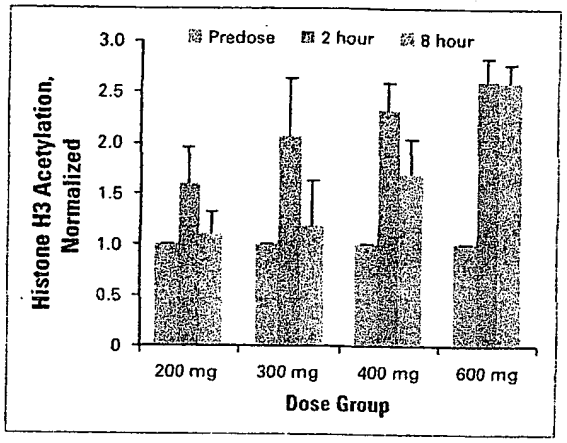

Fig 3. Average histone H3 acetylation by dose group. Histones were isolated at the times indicated following ingestion of suberoylanilide hydroxamic acid and enzyme-linked immunosorbent assays performed as described in Patients and Methods. The number of patients analyzed in the 200-, 300-, 400-, and 600-mg groups was five, three, seven, and three, respectively. Each determination was performed in triplicate and the error bars represent the standard error of the mean.

and solid tumor patients. This is in contrast to the intravenous study of SAHA that showed that myelosuppression limited the dose escalation in patients with hematologic malignancies and there was a three-fold difference in the MTD between solid tumor and hematologic patients.[9] The more extensive prior therapy hematologic patients received may account for these differences. The DLTs with oral SAHA were not related to prior therapy or the type of underlying malignancies but remained relatively unpredictable within treatment cohorts. The fatigue could occur rapidly and was associated with anorexia, dehydration, diarrhea, and a feeling of dyskinesia. Once the oral SAHA was discontinued, these toxicities resolved quickly within 4 to 7 days. The etiology of the fatigue is not known, but their rapid resolution after withdrawing the SAHA suggests a readily reversible metabolic process.

In this study, the majority of the patients were treated at MTD or above and once on a tolerable dose, patients could be treated for prolonged periods of time, in some cases for over 2 years without loss of the biologic effect. Chronic adverse effects (fatigue, renal insufficiency, and weight loss) seen in long-term treated patients were generally mild to moderate and were reversible on discontinuation of the study drug, suggesting that chronic administration of SAHA is feasible and safe.

The altered PK profile of oral SAHA as compared with IV SAHA is likely to have contributed to the differences in toxicity, prolonged biologic effect, and the clinical outcomes in patients. As with the IV formulation of SAHA, the extent of exposure after oral SAHA administration was linear in dose ranges from 200 to 600 mg.[9] Peak concentrations were substantially lower after oral administration, but there was a two- to three-fold increase in the apparent half-life when compared to the IV administration. Oral SAHA plasma concentrations could be detected

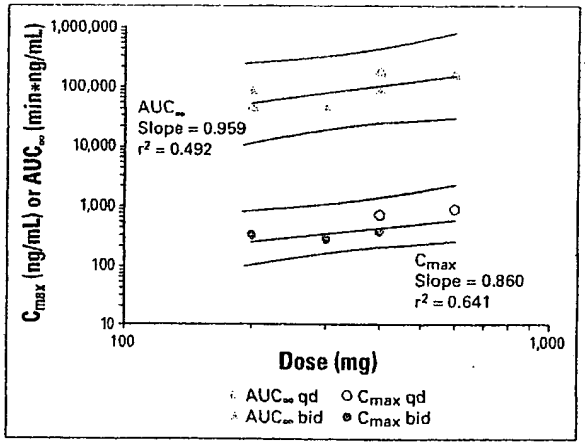

Fig 2. Relationship between area under the curve to infinity (AUC_∞) and maximum concentration (C_{max}) and dose on cycle 1/day 8 after administration of suberoylanilide hydroxamic acid under fasting conditions. qd, every day; bid, twice a day.

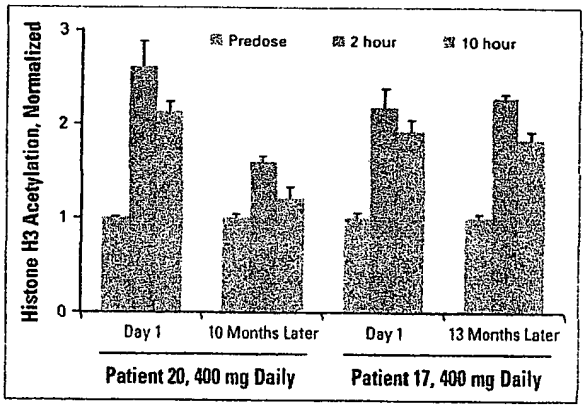

Fig 4. Long-term evaluation of peripheral-blood mononuclear cells for histone H3 acetylation in patients.

Table 5. Patients on Study ≥ 4 Months

Tumor	SAHA dose (mg)	No. of Prior Systemic Therapy	Best Response	On-Study Duration (months)
Solid tumor				
Thyroid	200 qd	1	SD	22
Renal cell*	400 qd	3	SD	4
Urothelial	200 bid	3	SD	10
Thyroid	200 bid	1	SD	28
Thyroid	300 bid	1	SD	28+
Mesothelioma	300 bid x 3 days/week	1	SD	8+
Mesothelioma	300 bid x 3 days/week	5	PR††	8
Mesothelioma†	300 bid x 3 days/week	2	SD	5
Mesothelioma‡	300 bid x 3 days/week	0	SD	5
Mesothelioma	300 bid x 3 days/week	1	PR††	6
Mesothelioma†	400 bid x 3 days/week	1	SD	10
Thyroid	400 bid x 3 days/week	1	SD	12
Laryngeal§	400 bid	1	PR	10
Renal cell¶	400 bid	4	SD	37+
Thyroid‖	400 bid	1	PR	34
Thyroid**	400 bid	2	SD	37+
Hematologic malignancy				
Hodgkin's lymphoma	400 qd	2	SD	10
Hodgkin's lymphoma	400 qd	8	SD	5
DLBCL (de novo)	600 qd	4	PR	5
DLBCL (transformed)	200 bid	3	SD	8
Cutaneous T-cell	200 bid	5	SD	4
DLBCL (transformed)	400 bid	8	CR	13

Abbreviations: SAHA, suberoylanilide hydroxamic acid, qd, every day; SD, stable disease; bid, twice a day, PR, partial response, DLBCL, diffuse large B-cell lymphoma; CR, complete response.
*400 qd for 7 weeks, 200 qd for 9 weeks.
†300 bid × 3 days/week for 4 weeks; 200 bid × 3 days/week for 14 weeks.
‡400 bid × 3 days/week for 33 weeks; 300 bid for 7 weeks.
§400 bid for 2 weeks; 400 qd for 36 weeks.
¶400 bid for 12 weeks; 400 qd for 136+ weeks.
‖400 bid for 4 weeks; 400 qd for 132 weeks.
**400 bid for 93 weeks; 400 qd for 53+ weeks.
††Unconfirmed.

at 10 hours postingestion at the higher dose levels while the plasma concentration of IV SAHA at similar doses were not detectable after 4 to 6 hours. This would suggest an absorption-rate–limited drug disposition in the GI tract and possibly hepatic recirculation to the GI tract.

As previously shown with intravenous SAHA using Western blot analysis, an increase in histone acetylation in PBMNCs was observed 2 hours post-therapy consistently in all patients evaluated and could persist for up to 10 hours after a single 400 mg or higher dose. This biologic effect paralleled the prolonged plasma concentrations of oral SAHA. An increase in acetylated histones was detected in patients who were on study for 6 months or longer, suggesting there is a sustained biologic effect over time.

Tumor regression and stable disease were observed in a wide range of patients with solid tumors and lymphomas. Four patients had confirmed CR and PR that occurred at the 400 mg bid and 600 mg qd dosing schedules and three of the 4 patients were treated on a twice daily regimen. This suggests that higher doses of SAHA with more prolonged daily exposure may be required for tumor regression. However, prolonged stable disease with minor tumor regression was seen at all dose levels and dosing schedules. Of particular interest was the clinical activity observed in patients with lymphoma and malignant mesothelioma. One CR and one PR were seen in patients with diffuse large B-cell lymphoma and two unconfirmed partial responses in patients with mesothelioma. Preclinical data suggest that HDACs play a critical role in the malignant transformation and cell differentiation[13-16] in these tumors and provides a rationale for developing HDAC inhibitors in these diseases. Of note in this study, 30% of the heavily pretreated patients had stable disease for 4 or more months and five patients remained on therapy for more than 2 years. Four of the five of these long-term patients had metastatic thyroid cancer which may have a more indolent course; however, all had objective disease progression before study entry. Thyroid cancer patients were initially accrued to this trial based on the data from Kitazono et al, showing that the HDAC inhibitor, depsipeptide, led to an increase in the expression to the Na+/iodine symporter that could result in an increase ^{125}I uptake in thyroid cells.[17] This could possibly lead to resensitizing RAI-refractory patients to RAI.[17,18] In this study, one patient did have an increase in the RAI scan post-therapy. Other plausible mechanisms for disease stabilization need to be investigated in thyroid cancer, since one patient with medullary thyroid cancer was also maintained on therapy for over 2 years.

In summary, this first study of oral SAHA demonstrated that SAHA could be administered safely for prolonged periods of time while maintaining the biologic effect of the drug and exhibiting a broad range of antitumor activity at multiple dose levels and dosing schedules. Future studies need to define the optimal dosing schedule and elucidate the biologic consequences of HDAC inhibition in patients. Currently, there are multiple phase II studies in patients with hematologic and solid tumor malignancies that are exploring the efficacy of daily and twice daily schedules that will help to determine the most optimal dosing regimen.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Employment: Judy H. Chiao, Aton Pharma; Paul Secrist, Aton Pharma; Victoria M. Richon, Merck & Company.

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase I and Pharmacokinetic Study of MS-275, a Histone Deacetylase Inhibitor, in Patients With Advanced and Refractory Solid Tumors or Lymphoma

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ABSTRACT

Purpose
The objective of this study was to define the maximum-tolerated dose (MTD), the recommended phase II dose, the dose-limiting toxicity, and determine the pharmacokinetic (PK) and pharmacodynamic profiles of MS-275.

Patients and Methods
Patients with advanced solid tumors or lymphoma were treated with MS-275 orally initially on a once daily × 28 every 6 weeks (daily) and later on once every-14-days (q14-day) schedules. The starting dose was 2 mg/m^2 and the dose was escalated in three- to six-patient cohorts based on toxicity assessments.

Results
With the daily schedule, the MTD was exceeded at the first dose level. Preliminary PK analysis suggested the half-life of MS-275 in humans was 39 to 80 hours, substantially longer than predicted by preclinical studies. With the q14-day schedule, 28 patients were treated. The MTD was 10 mg/m^2 and dose-limiting toxicities were nausea, vomiting, anorexia, and fatigue. Exposure to MS-275 was dose dependent, suggesting linear PK. Increased histone H3 acetylation in peripheral-blood mononuclear-cells was apparent at all dose levels by immunofluorescence analysis. Ten of 29 patients remained on treatment for ≥ 3 months.

Conclusion
The MS-275 oral formulation on the daily schedule was intolerable at a dose and schedule explored. The q14-day schedule is reasonably well tolerated. Histone deacetylase inhibition was observed in peripheral-blood mononuclear-cells. Based on PK data from the q14-day schedule, a more frequent dosing schedule, weekly × 4, repeated every 6 weeks is presently being evaluated.

J Clin Oncol 23:3912-3922.

INTRODUCTION

Histone deacetylases (HDACs) regulate gene expression.[1-4] HDAC inhibitors (HDIs) have induced gene activation, cellular differentiation, cell growth arrest, and apoptosis in cancer cells.[4] MS-275 is an orally active synthetic pyridyl carbamate HDI.[5] In the National Cancer Institute's (NCI's) 60 cell-line screen, MS-275 displays a unique pattern of cytotoxicity with potent antiproliferative activity.[6] Microarray analysis suggests MS-275 promotes gene expression favoring growth arrest and differentiation with significantly increased expression of antiproliferation genes such as p21 and transforming growth factor-beta type II receptor, as well as induction of the maturation marker gelsolin.[5-8] In vivo tumor volume reduction was observed in gastric, epidermoid, pancreatic, colon, ovarian, and non–small-cell lung cancer (NSCLC) xenograft models on an oral daily × 28

schedule,[5] as well as in myeloma, promyelocytic leukemia, and small-cell lung cancer models.[6]

Preclinical pharmacology studies indicated that MS-275 peak plasma concentration (T_{max}) was 30 to 40 minutes (administered orally) with a half-life ($T_{1/2}$) of approximately 1 hour, similar in rats, mice, and dogs. Approximately 85% of the drug was bioavailable with oral administration. The dose-limiting toxicity (DLT) was myelosuppression in all species. In an oral daily × 28-day schedule, the maximum tolerated dose (MTD) was 6 mg/m^2 for dogs and 18 mg/m^2 for rats. Adverse events were usually observed during the third and fourth week of dosing. In vitro, human bone marrow sensitivity to MS-275 was similar to rats.[9]

Based on animal data, we conducted a phase I, open-label, single-arm, dose-escalation study in advanced solid tumor and lymphoma patients, with the primary objectives of defining MTD, DLT, and an optimal dose and schedule for a phase II study. Other objectives were to determine safety and tolerability, pharmacokinetic and pharmacodynamic profiles, the ability of MS-275 to affect its target in a surrogate tissue, and antitumor activity. The results of the daily schedule and every-14-day (q14-day) schedule are included in this report.

PATIENTS AND METHODS

Patients

Inclusion criteria were as follows: (1) pathologically confirmed malignancy that was metastatic or unresectable, and for which standard curative or palliative measures did not exist or would likely not be effective; (2) an Eastern Cooperative Oncology Group performance status ≤ 2, with no recent (within 2 months) weight loss of >10% of average body weight; (3) life expectancy greater than 3 months; (4) age ≥ 18 years; (5) leukocytes ≥ 3,000/μL, absolute neutrophil count ≥ 1,500/μL, platelets ≥ 100,000/μL, creatinine within normal limits or measured creatinine clearance ≥ 60 mL/min/1.73m^2, total bilirubin ≤ 1.5 × upper limit of normal, AST /ALT ≤ 2.5 × upper limit of normal, adequate oral intake and serum albumin > 75% of lower limit normal; and (6) able to give written consent, willing to self administer and document the doses of MS 275 as needed, and able to return to NCI for follow-up.

Exclusion criteria were as follows: (1) those who had received prior anticancer therapy (chemotherapy, radiotherapy, vaccines, and hormone therapy with the exception of gonadotropin hormone-releasing hormone agonists) within 4 weeks of study entry (6 weeks for nitrosoureas or mitomycin C, 8 weeks for UCN-01), or those who have not recovered from adverse events (reduced to grade 2 or less) as a result of agents administered more than 4 weeks earlier; (2) known brain metastases; (3) history of allergic reactions attributed to compounds of similar chemical or biologic composition to MS-275; (4) uncontrolled intercurrent illness; (5) pregnant or lactating women; (6) men and women of reproductive potential without adequate contraception; (7) known HIV; (8) gastrointestinal conditions that might predispose for drug intolerability or poor drug absorption; and (9) major surgery within 21 days of study entry, intercurrent radiation, chemotherapy, immunotherapy, or hormonal therapy (except for gonadotropin hormone-releasing hormone agonists).

Dosage and Dose Escalation Scheme

The initial human dosing schedule was daily oral administration for 28 days and 14-day recovery period, constituting a 42-day cycle. MS-275 was administered with food, owing to evidence of enhanced bioavailability from animal studies in the fed state. A starting dose of 2 mg/m^2 (1/10th rat MTD) with an accelerated dose escalation at increments of 100% and single patient per dose level was planned.

Due to unexpected toxicities, the subsequent dosing schedule was changed to once orally every 14 days. Administered in the fed state, the starting dose level was again 2 mg/m^2, using a modified Fibonacci dose escalation scheme (three to six patient cohorts) with a dose escalation increment of 2 mg/m^2 without intrapatient dose escalation.

DLT was defined as first course adverse events ≥ grade 3 nonhematologic or ≥ grade 4 hematologic toxicity. The MTD was defined as one dose level below the dose at which ≥ two of six patients experience DLT.

Dose reduction by one level was applied for the occurrence of either grade 3 nonhematologic toxicity, grade 4 hematologic toxicity, persistent (≥ 2 weeks) grade 2 nonhematologic toxicity, or per the investigator's assessment. For dose level 1, 25%, 50%, and 75% decrease in starting dose was the order of dose reduction. No limitation for the number of dose reductions was chosen.

Safety and Efficacy Measures

At study entry, history, physical examination, laboratory studies (CBC, electrolytes, creatinine, blood urea nitrogen, total and direct bilirubin, ALT, AST, alkaline phosphotase, uric acid, prothrombin time, partial thromboplastin time, and urinalysis), computed tomography scan, and chest x-ray and ECG were performed. Clinical assessments, including a physical examination and adverse event evaluation, were conducted at each follow-up. Adverse events were graded by the NCI Common Toxicity Criteria (version 2.0). Computed tomography scans and staging was performed every 6 weeks for the q14-day schedule. Disease-specific staging techniques, such as bone marrow aspirate and biopsy, flow cytometry, cutaneous lesion photography, or bone scan were used as indicated. Response evaluations used the Response Evaluation Criteria in Solid Tumors[10] and the Cheson criteria[11] for lymphoma. Multiple-gated acquisition (MUGA) scans were obtained on the q14-day schedule at base line, before course 2, and at each restaging. Laboratory studies (CBC with differential, chemistry 20, prothrombin time, and partial thromboplastin time) were performed on days 1, 3, 5, and 7 and repeated weekly. Twenty-four-hour urine clearance, albumin, protein, uric acid, and electrolytes were performed at baseline, and on days 3 and 13.

Pharmacokinetic Studies

Blood samples (6 mL) were collected in sodium heparin tubes at 0, 2, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours after the first dose. Following initial pharmacokinetic evaluation of data obtained from the first two dose levels, the sampling also included 30 minutes and 1 hour. Samples were immediately centrifuged at 3,000 g for 10 minutes at 4°C and then plasma was divided into two aliquots of at least 1 mL and frozen at −70°C until the time of analysis. Plasma samples were assayed by a specific and sensitive high-performance liquid chromatographic assay with

mass-spectrometric detection.[12] The lower limit of quantitation of this assay is 0.50 ng/mL, with values for precision and accuracy of ≤ 5.58 and ≤ 11.4% relative error, respectively.[12]

Estimates of pharmacokinetic parameters for MS-275 were derived from individual concentration-time data sets by noncompartmental analysis using the software package WinNonlin version 4.0 (Pharsight Corporation, Mountain View, CA). The peak plasma concentrations and the time to peak concentrations were the observed values. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal method from time zero to the time of the final quantifiable concentration (AUC_{tf}). The AUC was then extrapolated to infinity (AUC_{inf}) by dividing the last measured concentration by the rate constant of the terminal phase (k), which was determined by linear-regression analysis of the final three or four time points of the log-linear concentration-time plot. The apparent oral clearance of MS-275 (CL/F) was calculated by dividing the administered dose by the observed AUC_{inf} and the $T_{1/2}$ was calculated by dividing 0.693 by k.

Statistical Analysis

Dose proportionality for MS-275 was assessed using a power model (ie, $AUC = \alpha \times dose^{\beta}$) where an ideal proportional model corresponds to $\beta = 1$ (ie, to a model of the form $AUC = \alpha \times dose$) and with the proportionality constant α. Deviations of β from 1 correspond to deviations from ideal dose proportionality. Interindividual differences in pharmacokinetic parameters were assessed by the coefficient of variation (CV), expressed as the ratio of the standard deviation to the observed mean (SD/M). All pharmacokinetic data are presented as mean ± SD except where otherwise indicated. The apparent CL/F and the $T_{1/2}$ were analyzed as a function of the MS-275 dose level using the Kruskal-Wallis' one-way analysis of ranks followed by the Dunn's multiple comparison test for identifying statistically significantly different groups. Variability in parameter estimates for MS-275 between cohorts of patients that did or did not experience DLT was evaluated by a one-sided Mann-Whitney U test for differences in medians after testing for normality and heteroscedasticity. One-way analysis of variance was performed to compare mean values using a two-sided Dunnett's test. Statistical calculations were performed using the Number Cruncher Statistical System 2001 series (J. L. Hintze, Kaysville, UT). The cut-off for statistical significance was considered at $P < .05$.

Pharmacodynamic Analysis

Immunocytochemical analysis of acetylated histone H3 was performed on peripheral-blood mononuclear cells (PBMCs), which were isolated from whole blood by centrifugation on Ficoll-Paque Plus (Amersham, Little Chalfont, United Kingdom), pelleted onto glass slides by cytocentrifugation, fixed in 95% ethanol/5% glacial EDTA for 1 minute and permeabilized with 0.2% Triton X-100 for 10 minutes at room temperature, then nonspecific binding sites were blocked by incubating the cells with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 hour at 4°C. Slides were incubated with polyclonal antiacetylated histone H3 antibody (Upstate Biotechnology, Lake Placid, NY) for 1 hour at 4°C, washed two times for 2 minutes with PBS, then incubated at 4°C for 1 hour with Cy3-conjugated goat antirabbit immunoglobulin (Molecular Probes, Eugene, OR), and washed again with PBS. Finally, slides were incubated with 4,6-diamidoino-2-phenylindole (Sigma, St Louis, MO) for 10 minutes at room temperature, rinsed quickly with water, air-dried, mounted using SlowFade (Molecular Probes), and imaged using a Zeiss Axiophot microscope interfaced with a CCD camera (Optronics Engineering, Goleta, CA). Positive controls were prepared by exposing healthy donor PBMCs to MS-275 in vitro. Buffy coats, provided anonymously as a byproduct of whole-blood donations from paid healthy volunteer donors through an international review board–approved protocol, were centrifuged on Ficoll-Paque Plus. Mononuclear cells were depleted of monocytes by adherence to plastic for 2 hours at 37°C and incubated with MS-275 in vitro for various times and at varying drug concentrations. Cells were processed for histone hyperacetylation in the same manner as patient samples. Images of PBMCs stained for acetylated histone H3 were imported into the Openlab image analysis program (Improvision, Coventry, United Kingdom) and histone acetylation levels were assessed using the Openlab quantification software.

RESULTS

General

Between April 5, 2001 and July 29, 2003, 31 patients have enrolled on the study (two on daily and 29 on the q14-day schedule). Thirty of them received MS-275 and were assessable. One patient with melanoma withdrew before receiving treatment owing to a disease complication. All patients (demographics in Table 1) had received prior therapy (median No. of prior treatments = 3): surgery (90%), prior chemotherapy (97%), radiotherapy (50%), and immunotherapy (50%).

Dose Escalation and DLT in Daily and q14-Day Schedule

The dose escalation experience for both the MS-275 daily and the q14-day schedules are summarized in Table 2.

Daily schedule. Two male patients were treated at the initial dose level of 2 mg/m^2 of the daily × 28 schedule. Both experienced DLT before the completion of the first cycle. DLTs observed were abdominal/epigastric pain in one patient, and cardiac arrhythmia (supraventricular tachycardia), elevated AST/ALT, hypotension, hypoalbuminemia, and hypophosphatemia in a second patient. All adverse events resolved within 2 to 3 weeks. Preliminary pharmacokinetic data from our initial two patients suggested that MS-275 had a 30- to 50-times longer half-life in humans than initially predicted from the animal models. This may explain the unforeseen toxicity observed in these two patients during the daily MS-275 schedule. Assessment of histone H3 and H4 acetylation indicated HDAC inhibition occurred after one dose of MS-275. To ensure safety, a q14-day dosing schedule was implemented.

q14-day schedule. A total of 28 patients have been treated on the q14-day schedule. The DLTs of MS-275 on a q14-day schedule were anorexia, nausea, vomiting, and fatigue. The MTD and recommended phase II dose of MS-275 for a q14-day schedule was 10 mg/m^2. As summarized in Table 2, the first patients with first course DLTs

Table 1. Demographics

Characteristic	No. of Patients
Total	31
Age, years	
Median	57
Range	36-76
Sex	
Male	19
Female	12
ECOG performance status	
0	7
1	21
2	3
Median	1
Tumor type	
Melanoma	6
Renal cell carcinoma	6
NSCLC	4
Sarcoma	4
Breast	2
Colorectal	2
Lymphoma	2
Cervix	1
Mesothelioma	1
Prostate	1
Small bowel	1
Thyroid	1
No. of prior chemotherapy	
0	1
1	6
2	10
≥ 3	14
Median	3
Range	0-20
No. of prior radiotherapy	
0	16
1	9
2	4
≥ 3	2
No. of prior immunotherapy	
0	16
1	9
2	6

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, non–small-cell lung cancer.

were observed at dose level 3 (6 mg/m^2). After five patients tolerated dose level 4 without DLT, dose escalation continued to level 5 (10 mg/m^2). One patient experienced similar DLTs at level 5 as had been seen at level 3. At dose level 6 (12 mg/ m^2), two patients experienced similar DLTs.

First course adverse events observed, either probably or possibly related to MS-275, are summarized in Table 3. There were no MS-275-related first course grade 4 adverse events There was only first course grade 4 adverse event (dyspnea) observed during the study, which occurred at dose level 6 (12 mg/m^2), was considered unrelated to the MS-275, and likely due to progression of metastatic mesothelioma. MS-275-induced fatigue, anorexia, nausea, and vomiting were observed as early as dose level 1 (2 mg/ m^2), and all were mild. With dose escalation, intensity of these toxicities gradually increased. Other less frequent drug-related toxicities included taste change, headache, diarrhea, flatulence, bloating, and reflux symptoms. Hematologic toxicities, such as thrombocytopenia and neutropenia, became more apparent at the higher dose levels (Table 3). Anemia was frequently noticed during the first course due to frequent pharmacokinetic and laboratory sampling, not related to MS-275.

Among drug-related biochemical abnormalities observed during the first course, the most frequently observed was hypoalbuminemia. Twenty-four hour urine analysis indicated there is no renal wasting of albumin, protein, or electrolytes. Clinically, no obvious gastrointestinal albumin loss was observed. The hypothesis that MS-275 may trigger inflammatory response, leading to albumin decrease, was examined by evaluating several patients' fibrinogen, C-reactive protein, and ferritin levels at baseline and after receiving MS-275, and no significant changes were found. No change in ACTH, cortisol, progestin, and estrogen was observed in patients who entered higher MS-275 dose levels (8, 10, and 12 mg/m^2) at 0 and 24 hours after the first dose. However, the prealbumin level was decreased after MS-275 administration, suggesting the possibility of production decline.

Symptomatic cardiac adverse events were not observed in patients who received q14-day MS-275. In 184 ECGs performed among 28 patients, there were no statistical or clinical adverse ECG interval (HR, PR, QRS, and QTc) effects observed. There were no ST–T wave changes from the

Table 2. Schedule, Dose Level, and Dose Administration

Dose Level and Schedule	Dose (mg/m^2)	Initial Patient No.	Total No. of Treatment Courses	No. of Patients With First Course DLT	DLTs
Every day × 28/42 days					
1	2	2	2*	2	See text
Every 14 days					
1	2	3	22 (4)	0	0
2	4	3	16 (4)	0	0
3	6	6	51 (8)	1	3†
4	8	5	22 (9)	0	0
5	10	6	30 (8)	1	3†
6	12	5	16 (5)	2	7‡

NOTE. Numbers in parentheses indicate total patients treated at dose level.
Abbreviation: DLT, dose-limiting toxicity.
*Due to DLTs, both patients' treatments were terminated before completing the first course.
†Anorexia, nausea, and vomiting.
‡Anorexia, nausea, vomiting, and fatigue.

Table 3. First Cycle Adverse Events Probably or Possibly Related to MS-275 at all Dose Levels (N = 28)

Adverse Events	All Grades		Grade 3
	No. of Patients	%	
Cardiovascular			
Sinus tachycardia	1	3	
Hematologic			
Anemia	8	29	
Leucopenia	6	21	
Lymphopenia	5	18	
Neutropenia	7	25	
Thrombocytopenia	10	36	
Gastrointestinal			
Anorexia	10	36	4
Constipation	2	7	
Diarrhea	2	7	
Dyspepsia	6	21	
Flatulence	3	11	
GI other	2	7	
Nausea	18	64	4
Stomatitis	1	4	
Vomiting	11	39	4
Laboratory			
Alkaline phosphatase	1	4	
Bilirubin	4	14	
Creatinine	2	7	
Hyperglycemia	3	11	
Hypermagnesemia	2	7	
Hypoalbuminemia	18	64	
Hypocalcemia	6	21	
Hypokalemia	1	4	
Hyponatremia	7	25	
Urinary electrolyte wasting	3	11	
General			
Allergic reaction	1	4	0
Dehydration	3	11	0
Depression	1	4	0
Fatigue	15	54	1
Fever	1	4	0
Headache	14	50	0
Infection w/o neutropenia	2	7	0
Libido	1	4	0
Middle ear infection	1	4	0
Muscle weakness	1	4	0
Myalgia	1	4	0
Nail changes	1	4	0
Sweating	1	4	0
Taste disturbance	8	29	0
Neuromuscular			
Neurosensory deficits	2	7	
Tremors	1	4	
Pain			
Abdominal pain	2	7	
Chest pain	2	7	
Pain other	1	4	
Pleuretic pain	1	4	
Respiratory			
Cough	1	4	
Rhinitis	1	4	

baseline. Ninety-one MUGA scans were performed. The mean left ventricular ejection fraction (LVEF) was 58.2% ± 1.62 (n = 28) at baseline and 58.7% ± 1.08 (n = 26) at follow-up. Twenty-six of 28 patients had both baseline MUGA and at least one follow-up MUGA. There were no statistically significant LVEF changes detected by the paired *t* test in these 26 patients ($P = .526$) or per individual dose level ($P = .106$ for 2 mg/m^2; $P = .350$ for 4 mg/m^2; $P = .133$ for 6 mg/m^2; $P = .951$ for 8 mg/m^2; $P = .201$ for 10 mg/m^2; and $P = .834$ for 12 mg/m^2).

A total of 157 courses of MS-275 were administered on the q14-day schedule (Table 2). Some cumulative adverse events caused treatment interruption with repeated MS-275 dosing. For example, grade 1 to 2 adverse events that occurred during early courses may progress to higher grades during later courses, requiring reduction in dose or dosing frequency. The dose reductions were frequent on dose levels higher than 8 mg/m^2, as noted in Figure 1 and Table 4. Frequent cumulative drug-related adverse events observed at or beyond course 2 were: anorexia, nausea, hypoalbuminemia, fatigue, headache, diarrhea, neutropenia, thrombocytopenia, leukopenia, and hypophosphatemia. Table 5 summarizes all drug-related occurring and grade 3 and grade 4 adverse events, occurring with a frequency of > 10% during the second course and beyond. Incidences of dose reduction after the second and subsequent courses of MS-275 are shown in Figure 1 and Table 4. On a q14-day schedule, the lowest doses (2 to 4 mg/m^2) are well tolerated, with ≤ 33% of patients going on to dose reduction, while in the 6 to 10 mg/m^2 dose range, ≥ 50% of patients ultimately required dose reduction. One patient with metastatic NSCLC, who had stable disease at the first restaging, withdrew himself from the study on the seventh day of course 4 and elected to receive standard chemotherapy (docetaxel 50 mg/m^2 every 3weeks). This patient developed a grade 4 neutropenia and leukopenia 8 days after receiving docetaxel, which was 16 days after the course 4 MS-275 dose. Taken together, the data of Tables 4 and 5 and Figure 1 suggest that while 10 mg/m^2 every 14 days was the formal MTD

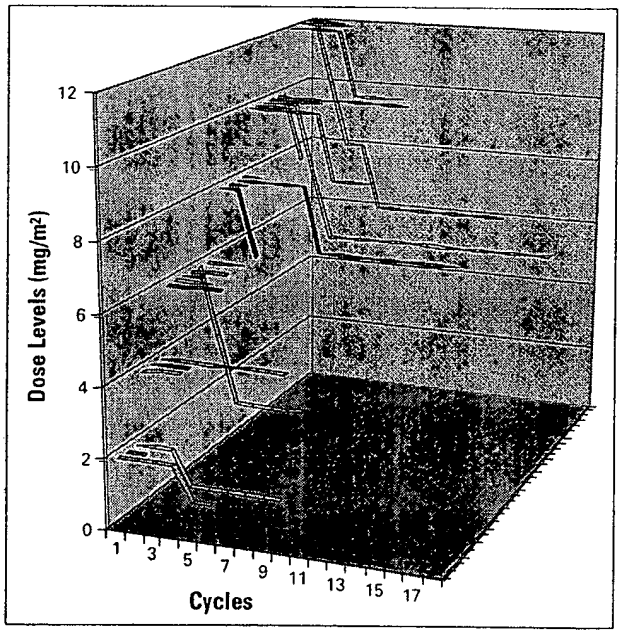

Fig 1. Patient dose reduction on an every-14-day schedule at each dose level. Each line represents a single patient started at their enrolled dose level and all subsequent dose modifications.

Table 4. No. of Patients Who Required Dose Reduction After Course 1

	2 mg/m^2	4 mg/m^2	6 mg/m^2	8 mg/m^2	10 mg/m^2	12 mg/m^2	Total No. of Patients
Course 1	3	3	6	5	6	5	28
Course 2	3	3	5	4	5	3	23
Course 3 or later	2	0	2	2	3	3	12

according to the definition of the protocol, in practice, titration of the tolerated dose to lower doses may be necessary during chronic or more frequent dosing.

With respect to reported HDAC inhibitor–induced immunosuppression, lymphopenia was observed during MS-275 administration. Only three instances of herpes simplex virus–positive stomatitis were found in patients receiving more than one course. A cutaneous T-cell lymphoma patient who had stable disease for over 4 months experienced one episode of herpes zoster recurrence in conjunction with clinical worsening of a skin bacterial infection.

Table 5. Frequent (≥ 10%) Adverse Events Observed During or After Course 2, Probably or Possibly Related to MS-275 (N = 23)

Adverse Events	All Grade		Grade 3	Grade 4
	No. of Patients	%		
Cardiovascular				
Left ventricular ejection fraction	3	13	0	0
Gastrointestinal				
Abdominal pain	5	22	0	0
Anorexia	13	56	1	0
Constipation	3	13	0	0
Diarrhea	8	35	3	0
Dyspepsia	6	26	0	0
Flatulence	3	13	0	0
Nausea	19	83	4	0
Stomatitis	3	13	0	0
Vomiting	7	30	1	0
General				
Arthralgia	4	17	0	0
Chest pain	4	17	0	0
Dehydration	5	22	0	0
Edema	4	17	0	0
Fatigue	23	100	3	0
Fever	5	22	0	0
Headache	12	52	0	0
Myalgia	7	30	0	0
Taste disturbance	10	43	0	0
Urine retention	2	9		
Hematology				
Anemia	6	26	0	0
Leukopenia	8	35	2	1
Lymphopenia	4	17	0	0
Neutropenia	17	74	3	1
Thrombocytopenia	14	61	1	0
Laboratory				
Alkaline phosphatase	3	13	0	0
Creatinine elevation	3	13	0	0
Hypercalcemia	4	17	0	0
Hyperglycemia	5	22	0	0
Hypernatremia	1	4	0	0
Hypoalbuminemia	11	48	1	0
Hypocalcemia	10	43	1	0
Hypomagnesemia	6	26	0	0
Hyponatremia	8	35	2	0
Hypophosphatemia	6	26	4	0
ALT	3	13		
Neuromuscular				
Muscle weakness	3	13		
Neurosensory	3	13		
Respiratory				
Dyspnea	4	22		

Responses

The treatment duration for each patient on the q14-day schedule is depicted in Figure 2. No complete or partial response was observed on the q14-day schedule. Fifteen cases of stable disease were observed with durations of 62 to 309 days. One cervical cancer patient, initially treated at 12 mg/m^2 had dose reduction thrice, continued on 6 mg/m^2 every 3 weeks after the fourth course, and sustained a 10-month period of stable disease. One NSCLC patient initially treated at 10 mg/m^2 also had dose reduction twice, with stable disease for 9 months. Two melanoma patients initially treated at 8 mg/m^2 and reduced to 6 mg/m^2 had stable disease for 4 and 5 months, respectively.

Pharmacokinetics

Pharmacokinetic studies were performed in 28 patients, with complete concentration-time profiles available for 27 patients. Figure 3 shows that plasma concentration

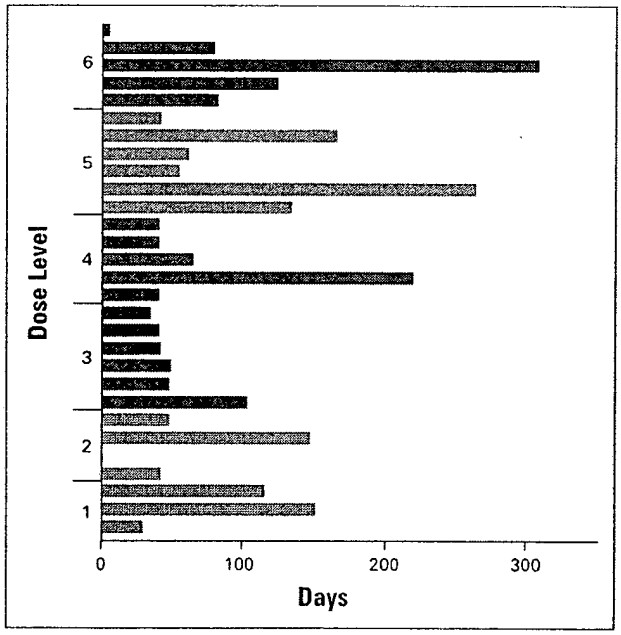

Fig 2. MS-275 treatment duration for patients on an every-14-day schedule. Treatment length ranged from 11 to 309 days. Although no complete response or partial response was observed on this regimen, 15 (52%) of 27 patients had stable disease ranging from 62 to 309 days.

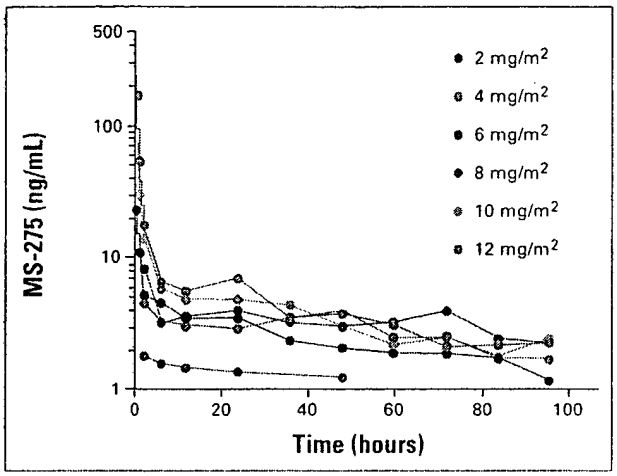

Fig 3. Concentration-time profiles of oral MS-275 at dose levels ranging from 2 to 12 mg/m^2 (n = 27). Data from the same dose levels were grouped and are presented as mean values (symbol) ± SE (error bar). The legend indicates each of the dose levels used.

versus time profiles of MS-275 were very similar at each dose level. The mean noncompartmental pharmacokinetic parameters of MS-275 ranging from 2 to 12 mg/m^2 are summarized in Table 6. Substantial interpatient variability in pharmacokinetic parameters was apparent at any dose level (CV for AUC, up to 53%). Similar variability was apparent in the CL/F (CV = 38.8%), implying varied systemic exposure to MS-275 during drug treatment. Absorption of the drug was highly variable with median T_{max} approaching 2 hours, with slow gastrointestinal uptake of MS-275 resulting in a T_{max} at 24 hours (n = 2), 48 hours (n = 1), and even 60 hours (n = 1), whereas a few patients exhibited T_{max} at 0.5 hours (n = 7), suggesting a rapid absorption and possible underestimation of the extent of drug uptake in these individuals.

Disappearance of MS-275 from the central plasma compartment was characterized by elimination in an apparent bi-exponential fashion, with an overall slow apparent CL/F of 17.4 ± 6.75 L/h/m^2. The estimated apparent terminal disposition half-life was relatively consistent in all patients, exhibiting a mean value of 51.74 ± 21.55 hours (CV = 41.7%). As a result of the slow clearance, MS-275 was detectable even 5 days after initial treatment in 19 of 27 patients.

The peak plasma concentrations, as well as the AUCs, increased in near proportion with increasing doses of MS-275 (Fig 4). The power model analysis indicated that the model poorly described the data, which estimates the parameter β was 0.517 ± 0.172 (R^2 = 0.323), while linear-regression analysis indicated near dose proportionality (R^2 = 0.556). The mean apparent CL/F of MS-275 was not significantly dependent on drug dose (P = .071) and the estimated $T_{1/2}$ was dose independent (P = .652). A preliminary analysis of pharmacokinetic-pharmacodynamic relationships for MS-275 suggests that drug exposure is significantly higher in patients experiencing DLTs (mean AUC, 517 ± 276 ng·h/mL, n = 4) compared with patients that had no DLT (280 ± 121 ng·h/mL, n = 23; P = .0477; Fig 5).

Analysis of PBMC Histone H3 Acetylation

Incubation of healthy donor PBMCs with MS-275 in vitro induced hyperacetylation of histone H3 (Fig 6A) in a concentration-dependent manner. Assayed at predosing and several time points postdosing, histone H3 hyperacetylation immunoflourescence images in PBMCs of two patients is shown (Fig 6A). The histone hyperacetylation quantified level was graphed for several patients (Figs 6B and C). The interpatient variability in histone hyperacetylation kinetics and intensities were apparent, as shown (Fig 6B: n = 7, 2 mg/m^2 and 4 mg/m^2; Fig 6C: n = 5, 10 mg/m^2). With limited sample size, there was no significant correlation between the AUC, AUC/dose, CL/F, C_{max}, C_{max}/dose, and the normalized change in histone H3 acetylation at 24 hours after the initial dose (data not shown). Histone hyperacetylation occurs at doses well below 10 mg/m^2, suggesting an optimal biologic effective dose may be much lower than the MTD defined by clinical toxicity.

DISCUSSION

To date, three subclasses of HDACs have been recognized. Class I, yeast RPD3 homologs (49-60 kD) include HDAC1,

Table 6. MS-275 Pharmacokinetic Parameters

Dose (mg/m^2)	No. of Patients	C_{max} (ng/mL) Mean	C_{max} (ng/mL) SD	AUC (ng·h/mL) Mean	AUC (ng·h/mL) SD	CL/F (L/h/m^2) Mean	CL/F (L/h/m^2) SD	$t_{1/2}$ (hours) Mean	$t_{1/2}$ (hours) SD	T_{max} Median	T_{max} Range
2	3	1.72	0.23	196.26	104.5	13.77	10.27	80.20	48.68	6	2–24
4	3	4.84	1.10	391.68	150.71	11.33	4.57	50.51	12.96	6	2–36
6	6	9.59	4.57	492.81	177.77	13.18	3.43	52.78	20.25	2	2–60
8	5	15.49	11.65	357.71	38.14	22.58	2.71	39.73	15.23	2	0.5–24
10	6	45.07	59.34	528.87	170.57	20.50	6.99	51.58	10.49	1.5	0.5–2
12	4	131.63	128.3	680.16	262.0	19.85	8.01	45.00	6.53	0.5	0.5–2
Grand mean						17.40	6.75*	51.74	21.55†		
Grand median										1.75	0.5–60

*P value for Kruskall-Wallis test (P = .071)
†P value for Kruskall-Wallis test (P = .652)

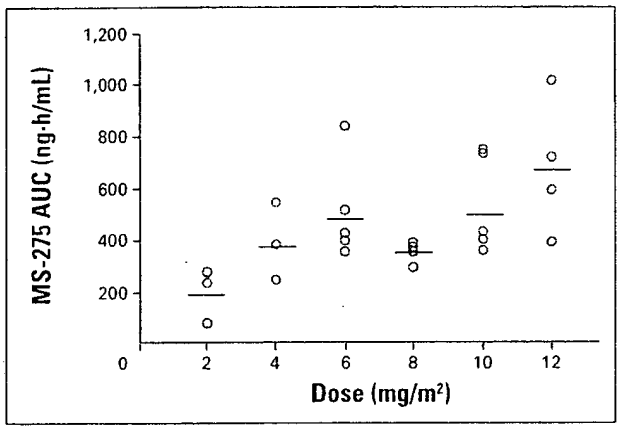

Fig 4. Effect of MS-275 dose on the area under the curve (AUC) at dose levels ranging from 2 to 12 mg/m^2 (n = 27). Each symbol represents data from an individual patient. Horizontal lines indicate the mean value for each dose group.

HDAC2, HDAC3, and HDAC8. Class II, yeast HDA1 homologs (> 100 kD) include HDAC4 (HDAC-A), HDAC5 (mHDA1), HDAC6 (mHDAC2), and HDAC7.[3,13-20] Class III are Sirt 1-7 and HDAC 11.[21] Different HDACs have been shown to associate with distinct transcriptional regulatory complexes[22-24] and different heterochromatic environments.[25] Nonhistone proteins, including cell structure elements (tubulin, HSP90), activators (p53, GATA-1), and transcription factors (TFIIE, TFIIF), were reported to be acetylated by histone acetyltransferases, suggesting that HDACs may regulate gene expression by deacetylation of nonhistone proteins.[26-29] HDACs may also participate in cell-cycle regulation, since Rb/E2F-mediated transcriptional repression involves recruitment of HDAC1 or HDAC2 by Rb.[30,31] HDIs present an exciting, novel approach for cancer therapy. They may augment gene-regulatory effects of coadministered DNA methyltransferase inhibitors.[33] Therefore, understanding HDI pharmacologic profiles as single agents is a prelude to constructing anticancer regimens to maximize gene expression modulation.

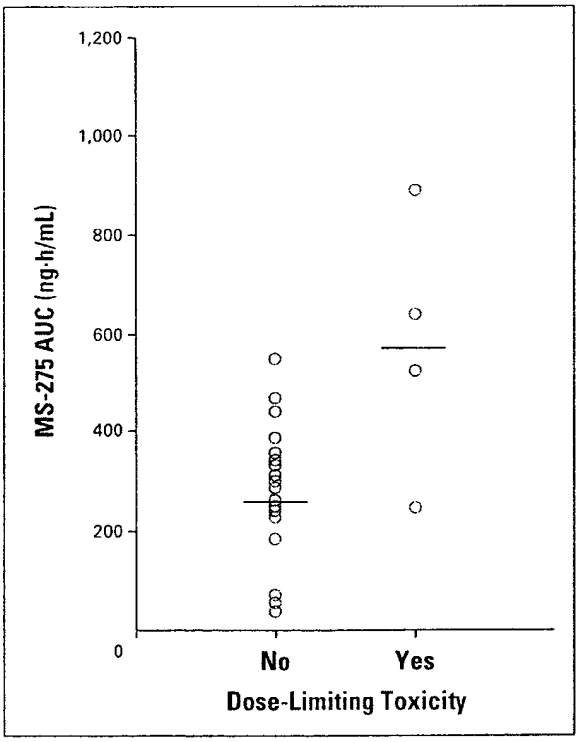

Fig 5. Comparative MS-275 area under the curve (AUC) from patients with and without a dose-limiting toxicity. Each symbol represents data from an individual patient.

Several classes of HDIs have been identified: (1) short-chain fatty acids—butyrates[33,34]; (2) hydroxamic acids—trichostatin A,[34,35] suberoylanilide hydroxamic acid (SAHA),[2] and oxamflatin[36]; (3) cyclic tetrapeptides containing a 2-amino-8-oxo-9, 10-epoxy-decanoyl (AOE) moiety—trapoxin A[37]; (4) cyclic peptides not containing the AOE moiety—depsipeptide and apicidin[38,39]; and (5) pyridyl carbamates—MS-275.[5] A number of HDIs induce differentiation, growth arrest, and/or apoptosis of tumor cells in vitro.[5,30-41] Some were able to inhibit growth of cancer cells in animal models.[5,42-46] A smaller number of these may be less toxic to the host and able to target tumors selectively.[5,47,48] Different HDIs appear to inhibit different HDAC subgroups. MS-275 inhibits HDAC 1, 3, 4, and 10.[49] None of the HDIs recognized to date are known to inhibit class III HDACs.[49]

Our data indicate that MS-275 can be given safely on a q14-day schedule, but not on a daily schedule in the dose range explored. Most frequent toxicities, including DLTs, were fatigue and gastrointestinal symptoms of nausea, vomiting, and anorexia for the q14-day schedule. Myelosuppression became apparent among cumulative adverse events related to MS-275. Unlike the daily schedule, the q14-day schedule had neither symptomatic nor diagnostic cardiac adverse events observed. It is clear that for MS-275 used on a q14-day schedule, the low to median dose range of 2 to 4 mg/m^2 is well tolerated among patients. MTD of 10 mg/m^2 provided peak plasma concentrations on average exceeding 75 ng/mL. This is above concentrations required in vitro and in vivo to induce significant growth inhibition in many models for various primary human tumors.[5,50] Although objective responses were not observed, 15 patients had stable disease while on a q14-day schedule.

Compared with the published data of other HDIs, neither grade 4 nonhematologic toxicities nor grade 2 or higher cardiac toxicities was observed on the q14-day schedule. Similarly, frequent nausea, vomiting, and dyspepsia were complications reported for sodium butyrate,[40,52] phenylbutyrate,[33,34,53] SAHA,[54] depsipeptide,[55] and tributyrin,[56,57] suggesting that the development of an oral HDAC inhibitor may be a challenge. The tolerable and reversible adverse event profile observed on a q14-day schedule does suggest that MS-275 might be a potentially

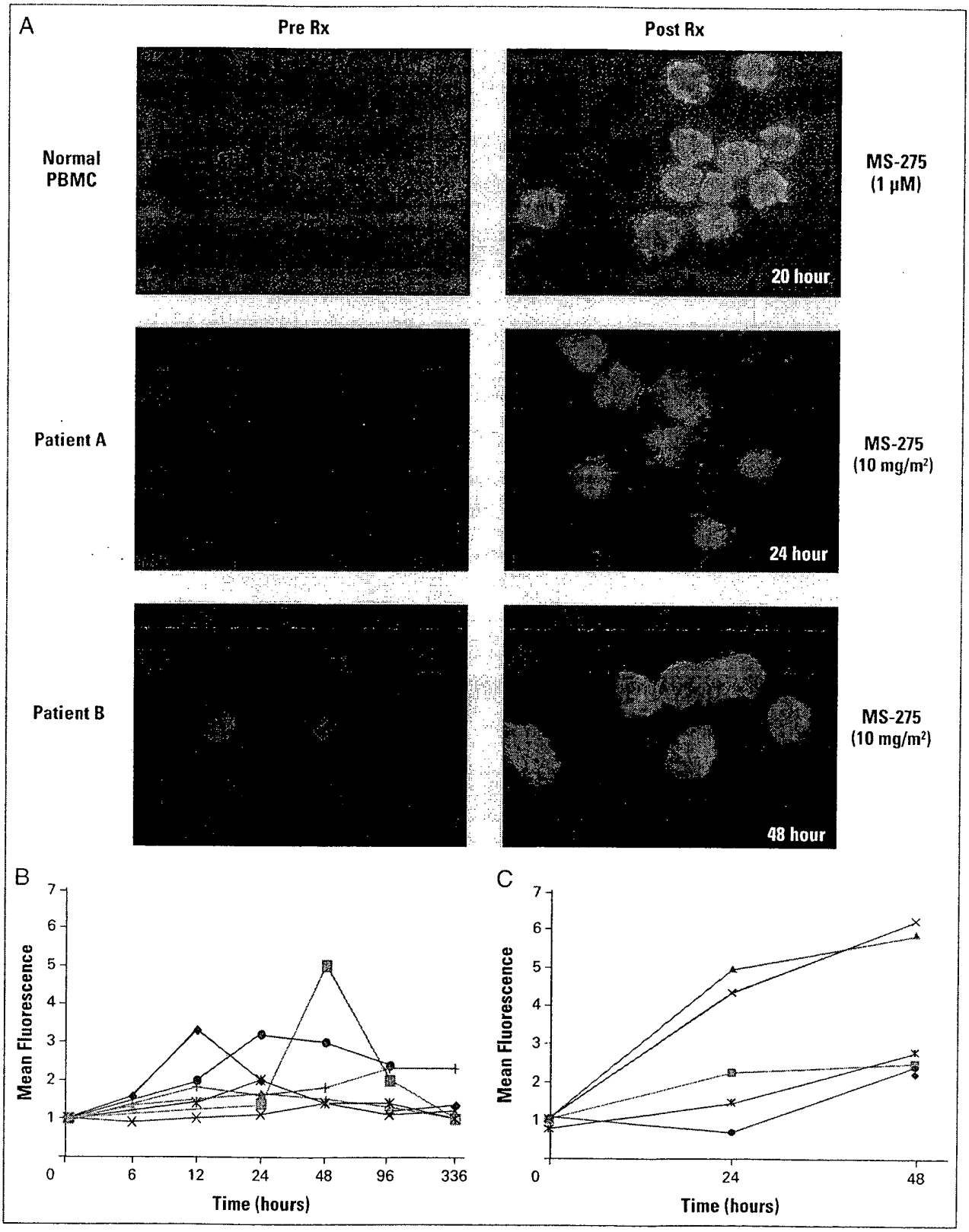

Fig 6. MS-275 induced histone H3 hyperacetylation. (A) Upper panel: healthy donor peripheral-blood mononuclear cells (PBMCs) incubated in vitro with MS-275 for 20 hours. Middle and lower panels: PBMCs from two patients treated with 10 mg/m^2 MS-275. (B) Mean fluorescence intensity of histone H3 acetylation in patients treated with 2 mg/m^2 MS-275; and (C) with 10 mg/m^2 MS-275.

well-tolerated chemotherapeutic agent. However, the q14-day schedule may not maintain a constant inhibition of HDAC activity. Presently, a weekly dosing schedule is being studied for tolerability and tumor response.

MS-275 displays a linear, dose-independent, pharmacokinetic behavior within the dose range studied (2 to 12 mg/m^2). Overall, drug absorption was rapid, and in some patients, the T_{max} was observed as early as 30 minutes, suggesting MS-275 might undergo rapid gastric absorption before reaching the small intestine. The disappearance of MS-275 was characterized by an apparent bi-exponential decline with a $T_{1/2}$ in plasma of approximately 50 hours, substantially longer than observed for MS-275 in laboratory animals (Schering AG, unpublished results). The basis for this long half-life in humans is possibly related to enterohepatic recirculation processes, suggested by the appearance of a second MS-275 peak around 24 to 48 hours after initial drug intake in several patients. Furthermore, the T_{max} observed at 24, 48, and 60 hours suggests a substantially longer normal gastrointestinal transit time. Any hypothetical recirculation is thus likely to mask the true disposition half-life of the free drug, as has been observed previously with many other agents.[58] Although other factors, including binding of the compound to plasma proteins such as human serum albumin and α_1-acid glycoprotein, may also influence the prolonged circulation of MS-275. We found that MS-275 is only $\approx$80% protein bound, and we did not find any greater binding affinity to albumin than to other proteins. Therefore, no significant clinical impact of protein binding on clearance is expected.

The observed variability in the pharmacokinetic behavior of MS-275, with an interpatient variability in the apparent CL/F of about 40%, is typical for cancer drugs administered orally.[59] Over the dose range studied, the MS-275 AUC demonstrated an apparent dose-independent behavior. Body-surface area correction did not account for the interpatient variability in clearance (38.8% *v* 39.5%), suggesting that body-surface area is not a significant predictor of oral MS-275 pharmacokinetics and that flat-dosing regimens might be applied without compromising overall safety profiles.

H3 acetylation in PBMCs provided a surrogate measure of HDAC inhibition after MS-275 administration. Our data demonstrate interpatient variability in the magnitude and kinetics of histone H3 hyperacetylation. Although MS-275 can induce histone H3 hyperacetylation in PBMCs in vivo, it is not clear whether histone H3 hyperacetylation is the most biologically relevant end point, nor is it known to what extent PBMCs reflect the MS-275 response in tumor cells in vivo. These should continue to be examined in relation to MS-275 and other clinically-relevant HCIs.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Consultant: Edward A. Sausville, Schering AG; Research Funding: Jane B. Trepel, Schering AG. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section of Information for Contributors found in the front of every issue.

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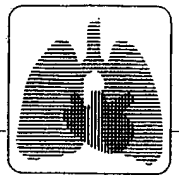

reviews

Surgical Treatment of Malignant Pleural Mesothelioma*

A Review

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Despite many years of clinical research, there is still no effective therapy for malignant pleural mesothelioma (MPM). Untreated, the prognosis is poor, with a median survival of < 1 year. Single-agent or combination chemotherapy as well as radiotherapy have not shown persistent improvements in response or survival. In general, MPM is a disease confined to the pleural cavity for a long time before metastasizing. Therefore, focus on local treatment seems rational. Surgical resection has been considered the mainstay of treatment by some. However, surgery alone results in high recurrence rates, and the survival benefit remains questionable. In recent years, the emphasis has been on surgery combined with adjuvant therapies. In this article, the present state of surgical management of MPM will be reviewed. ***(CHEST 2003; 123:551–561)***

Key words: adjuvant therapy; mesothelioma; pleural cavity; review; surgery

Abbreviations: EPP = extrapleural pneumonectomy; IMIG = International Mesothelioma Interest Group; MPM = malignant pleural mesothelioma; PDT = photodynamic therapy

Malignant pleural mesothelioma (MPM) is an aggressive tumor of the pleura. Presenting symptoms are dyspnea and chest pain in the majority of patients. Coughing, fatigue, and weight loss are less frequently observed.[1,2] In general, MPM is a disease confined to the pleural cavity for a long time before metastasizing.[3] The most common features are pleural thickening, nodularity, and pleural effusion. The growth pattern is characterized by involving the entire pleura and interlobular space.[2] Malignant seeding along tracts of cytology or biopsy needles, chest tubes, thoracoscopy trocars, and surgical incisions is a common complication of diagnostic and therapeutic procedures in patients with MPM.[4] In Western Europe, 5,000 patients die of mesothelioma each year.[5] In the last decades, the incidence has increased twofold in the Netherlands, and it is expected to reach its maximum in the year 2020.[6] The association with asbestosis is well known. In approximately 80% of MPM, an exposure to asbestos is reported. The latency period is between 20 years and 30 years.[7,8] Recently, a virus has become a suspected agent too.[9] Simian virus 40, a DNA tumor virus, has the potential to induce mesothelioma in hamsters and is reported to be identified in a number of patients with MPM.[9] However, there are still discussions ongoing about the potential of simian virus 40 to induce MPM in humans. The prognosis of patients with MPM is poor; untreated, the median survival is 9 months.[10,11]

Systemic chemotherapy results in partial responses of between 15% and 20%; complete responses are rare.[12–15] Radiotherapy as single treatment modality, administered with curative intent, is considered not feasible because of the large target volume and the dose-limiting toxicity of the adjacent organs and structures. Radiotherapy is considered useful for palliation and prevention of seeding after invasive diagnostic procedures.[4,16,17]

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Surgical resection has been considered the mainstay of treatment by some. However, it is almost impossible to achieve a microscopically complete resection with surgery alone because of the anatomy of the pleura and the property of MPM to infiltrate the underlying and neighboring structures.[18] Surgery alone is associated with a high recurrence rate. Recently, most efforts have been put in the combination of cytoreductive surgery with some form of adjuvant therapy.[19,20]

In this article, we review the surgical management of MPM. The different techniques and treatment outcome of surgery alone are described. Thereafter, emphasis is given to the adjuvant therapies.

Materials and Methods

A systemic literature study was performed to identify all relevant articles until October 2001. A MEDLINE search was performed with key words focused on MPM. Studies with < 10 patients were not included unless they showed very interesting results. When there were several reports of the same institute including the same cohort of patients with the same treatment, we listed only the most recent report. A statistical analysis of all reviewed articles was not possible due to the lack of randomized studies, the small patient groups, and the diversity of patient groups and methods.

Staging

Staging is important in the treatment of MPM.[21] Different staging systems are used (Table 1).[22–24] To stage accurately, several staging methods are used. Thoracoscopy, CT, MRI, and laparoscopy can identify the T status.[25,26] CT compared to MRI has nearly equivalent diagnostic accuracy. MRI is superior in imaging diaphragmatic muscle involvement, endothoracic involvement, and revealing solitary foci of chest wall invasion.[27] To accurately determine the nodal status is more often a problem. CT has a low accuracy regarding lymph nodes.[27] Mediastinoscopy is useful; however, 25% of the patients with MPM have nodal involvement confined to areas such as peridiaphragmatic and internal mammary regions not accessible to the mediastinoscopy.[28] Positron emission tomography seems to be useful to determine the extent of tumor.[29] Unfortunately, correct staging is only possible during operation in a substantial number of patients. The accuracy of preoperative CT scans to determine the stage correctly varies, but is reported as low as 30%.[1,30] The intraoperative tumor load is associated with outcome of MPM, and large volumes are associated with nodal spread.[31]

Prognostic Factors

In studies of Rusch and Venkatraman[28] and Sugarbaker et al,[32] the stage, histology, and adjuvant therapy, but not type of resection, were significant prognostic factors. Stage is a clear prognostic factor. Rusch and Venkatraman[28] reported a median survival after surgery with adjuvant therapy of 29.9 months for stage I, 19 months for stage II, 10.4 months for stage III, and 8 months for stage IV (International Mesothelioma Interest Group [IMIG] staging). Another study showed that when the visceral pleura was intact, the median survival was 32.7 months.[33] The node status alone has also prognostic significance with survival advantage for lymph node-negative patients.[23] Sarcomatous MPM shows a worse survival than the epithelial type.[23,32] Rusch and Venkatraman[28] found that female patients show better survival than male patients; however, Sugarbaker et al[32] could not confirm this. The type of resection, *ie*, extrapleural pneumonectomy (EPP) or a pleurectomy/decortication, did not have impact on survival in the study of Rusch and Venkatraman.[28] However, both procedures were performed only if they led to complete resection of all gross tumor. In patients with bulky tumor or confluent pleural tumor, an EPP was necessary to achieve complete resection.[28]

Results

Surgery Alone

Pleurectomy/Decortication: The technique of pleurectomy has been well described.[34] After a posterolateral thoracotomy, an extrapleural plane between the parietal pleura and the endothoracic fascia is entered. The dissection proceeds in a superior direction toward the apex over the posterolateral aspect of the chest wall. The dissection is continued to inferior and posterior. When the pleura and the lung are completely mobilized in the upper part of the thoracic cavity, the superior and posterior hilar structures of the lung are well exposed. After stripping or partial resection of the posterior pericardium, the dissection proceeds toward the posterior diaphragmatic sulcus. If there is only superficial involvement, dissection is performed through the diaphragmatic muscle, avoiding entering the abdomen; otherwise, a part of the diaphragm is removed. The *en bloc* specimen is mobilized back to the pericardium medially. When the dissection is completed to the hilar structures, the parietal pleural is opened and decortication of the visceral pleura is performed. The pericardium and diaphragm are eventually reconstructed.

The mortality of this procedure is limited (1 to 2%), when performed in specialized centers.[34,35] The most common complication is prolonged air leakage, occurring in 10% of cases. Other reported complications are pneumonia, empyema, and hemorrhage.[2] Pleurectomy and decortication are reported to be effective in controlling pleural effusion. The median survival reached by this procedure is reported in different studies between 9 months and 20 months (Table 2).

The technical problem is the difficulty of separating the visceral pleura from the lung parenchyma. This results frequently in incomplete resection.[3] After pleurectomy/decortication, Hilaris et al[44] reported that residual tumor was left behind in 78% of the patients, most frequently on the visceral pleura. The most common site of recurrence is the ipsilateral hemithorax.[34] In recent years, pleurectomy/decortication studies all included adjuvant therapy.

Table 1—*Staging Systems*

Stage	Description
Butchart et al[22]	
I	Tumor confined to ipsilateral pleura, lung, and pericardium
II	Tumor invading chest wall or involving mediastinal structures, *eg*, esophagus, heart, opposite pleura; lymph node involvement within the chest
III	Tumor penetrating diaphragm to involve peritoneum directly; lymph node involvement outside the chest
IV	Distant blood-borne metastases
Sugarbaker et al[23]	
I	Disease confined to within capsule of the parietal pleura: ipsilateral pleura, lung, pericardium, diaphragm, or chest-wall disease limited to previous biopsy sites
II	All stage I with positive intrathoracic (N1 or N2) lymph nodes
III	Local extension of disease into chest wall or mediastinum; heart, or through diaphragm, peritoneum; with or without extrathoracic or contralateral (N3) lymph node involvement
IV	Distant metastatic disease
IMIG[24]	
T primary tumor and extent	
T1	
a	Tumor limited to ipsilateral parietal pleura, including mediastinal and diaphragmatic pleura; no involvement of the visceral pleura
b	Tumor involving the ipsilateral parietal pleural, including mediastinal and diaphragmatic pleura; scattered foci or tumor also involving the visceral pleura
T2	Tumor involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • Involvement of diaphragmatic muscle • Confluent visceral pleura (including the fissures) or extension of tumor from visceral pleura into the underlying pulmonary parenchyma
T3	Describes locally advanced but potentially resectable tumor; tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • Involvement of the endothoracic fascia • Extension into the mediastinal fat • Solitary, complete resectable focus or tumor extending into the soft tissues of the chest wall • Nontransmural involvement of the pericardium
T4	Describes locally advanced technically irresectable tumor; tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • Diffuse extension or multifocal mass of tumor in the chest wall, with or without associated rib destruction • Direct transdiaphragmatic extension of the tumor to the peritoneum • Direct extension of tumor to the contralateral pleura • Direct extension of tumor to one or more mediastinal organs • Direct extension of tumor into the spine • Tumor extending through the internal surface of the pericardium with or without a pericardial effusion or tumor involving the myocardium
N lymph nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases in ipsilateral bronchopulmonary or hilar lymph nodes
N2	Metastases in the subcarinal or the ipsilateral mediastinal lymph nodes, including the ipsilateral internal mammary nodes
N3	Metastases in contralateral mediastinal, contralateral internal mammary, ipsilateral, or contralateral supraclavicular scalene lymph nodes
M metastases	
Mx	Presence of distant metastases cannot be assessed
M0	No (known) metastasis
M1	Distant metastasis present
Stage grouping	
I	
a	T1aN0M0
b	T1bN0M0
II	T2N0M0
III	Any T3M0, any N1M0, any N2M0
IV	Any T4, any N3, any M1

Table 2—*Studies With Pleurectomy Alone (Only Studies With > 10 Patients Are Listed)**

Source	Treatment	Year	Patients, No.	Median Survival, mo	2-yr Survival, %	Mortality, %
Chahinian et al[36]	Pleurectomy	1982	30	13	27	0
Brenner et al[37]	Pleurectomy	1982	69	15	NA	NA
Law et al[38]	Pleurectomy	1984	28	20	9	11
Chailleux et al[39]	Pleurectomy	1988	29	14	NA	NA
Ruffie et al[11]	Pleurectomy	1989	63	10	NA	0
Brancatisano et al[40]	Pleurectomy	1991	45	16	21	2
Rusch et al[41]	Pleurectomy	1991	26	10	20	NA
Allen et al[42]	Pleurectomy†	1994	56	9	9	5
Soysal et al[35]	Pleurectomy†	1997	100	17	NA	1
Ceresoli et al[43]	Pleurectomy	2001	38	13	NA	NA

*NA = not available.
†Some patients received adjuvant therapy.

EPP: EPP is a procedure consisting of *en bloc* resection of the lung, visceral and pleural pleura, pericardium, and ipsilateral diaphragm with reconstruction of the pericardium and diaphragm.[45] After a posterolateral thoracotomy through the sixth intercostal space, a dissection between the chest wall and parietal pleura is started. A blunt dissection with fingers appears to work best. After reaching the apex of the chest, the dissection will be proceeded to inferior (diaphragm). The diaphragm is opened while aiming to preserve the peritoneum. The whole diaphragm is removed. Next, the pericardium is resected. The specimen is then elevated, and the dissection continues to the hilar structures. After stapling the vessels and the bronchus, the specimen is removed. A pericardial fat pad can be placed over the bronchial stump. Reconstruction of the diaphragm and pericardium is the last stage of the procedure. In the patch to reconstruct the pericardium, fenestrations are made to prevent cardiac tamponade.

The mortality of this procedure has decreased in the last decades from 30 to < 5% when performed in specialized centers and in selected patients.[46,47] Causes of death are respiratory failure, myocardial failure, and pulmonary embolus.[2] The reported morbidity is considerable, mostly between 25% and 50%.[15,46] Twenty-four percent of the patients undergoing pneumonectomy showed cardiac supraventricular dysrhythmias with a peak incidence at 3 to 4 days after resection.[48] Patients are at risk of postoperative pneumonia, and the development of a bronchopleural fistula is reported in 10 to 20%, especially right-sided EPP.[2,23] Median survival after EPP is ranges from 9 to 19 months (Table 3).

EPP is performed for locally advanced disease, usually in patients with confluent visceral pleural tumor not separable from the lung and a partially or totally fused pleural space. Compared with pleurectomy/decortication, a lower recurrence rate has been reported (10% after EPP vs 52% after pleurectomy).[41,50] However, relapses in distant sites are more frequently seen than in the pleurectomy group, especially in adjacent cavities.[51] Because of operative deaths, residual tumor, local recurrence, and metastatic disease, EPP has not gained wide acceptance as treatment on its own.[41] There does not seem to be a survival benefit for patients undergoing EPP in comparison to patients undergoing pleurectomy.[41]

Surgery alone is associated with a high recurrence rate, and therefore adjuvant therapy seems useful.[19,20] Studies performed with the combination of surgery and adjuvant treatment are listed in Table 4.

Table 3—*Studies With EPP Alone (Only Studies With > 10 Patients Are Listed)**

Source	Treatment	Year	Patients, No.	Median Survival, mo	2-yr Survival, %	Mortality, %
Worn[49]	EPP	1974	62	19	37	NA
Butchart et al[22]	EPP	1976	29	10	9	31
Ruffie et al[11]	EPP	1989	23	9	NA	13
Rusch et al[41]	EPP	1991	20	10	33	15
Allen et al[42]	EPP†	1994	40	13	23	8

*See Table 2 for expansion of abbreviation.
†Some patients received adjuvant therapy.

Table 4—*Studies With Surgery and Adjuvant Therapy (Only Studies With > 10 Patients Are Listed)**

Source	Treatment	Year	Patients, No.	Median Survival, mo	2-yr Survival, %	Mortality, %
Surgery and emphasis on external radiotherapy						
McCormack et al[52]	S, XRT, C	1982	18†	16	NA	2
Hilaris et al[44]	S, XRT, B	1983	41	21	41	0
Alberts et al[53]	S, XRT, C	1988	26	11	NA	NA
Mattsson et al[54]	S, XRT, C	1992	100	8	20	NA
Sugarbaker et al[32]	S, XRT, C	1996	120	21	45	5
Rusch et al[55]	S, XRT	2001	61	17	NA	8
Surgery and emphasis on systemic chemotherapy						
Davalle et al[56]	S, C, XRT	1986	17	18	24	9
Huncharek et al[57]	S, C	1996	21	24	NA	NA
Hastürk et al[58]	S, C, I	1996	20	12	15	NA
Ceresoli et al[43]	S, C	2001	16	14	NA	NA
Surgery and emphasis on intrapleural chemotherapy						
Rice et al[59]	S, IPC, C	1994	19	13	25	5
Rusch et al[60]	S, IPC, C	1994	27	18	40	4
Sauter et al[61]	S, IPC, C	1995	13	9	15	8
Lee et al[62]	S, IPC	1995	15	12	16	0
Colleoni et al[63]	S, IPC, C	1996	20	12	34	0
NCI‡	S, IPC	2001	20	11	NA	0
Surgery and emphasis on photodynamic therapy						
Pass et al[64]	S, PDT, C, I	1997	25	14	NA	4
Moskal et al[65]	S, PDT	1998	40	15	23	7
Schouwink et al[66]	S, PDT	2001	28	10	NA	11

*S = surgery; XRT = external beam radiation therapy; B = brachytherapy; C = systemic chemotherapy; I = immunotherapy; IPC = intrapleural chemotherapy. See Table 2 for expansion of other abbreviation.

†Epithelial mesothelioma only.

‡Data from the Netherlands Cancer Institute (NCI) [not published].

Surgery and Emphasis on External Radiotherapy

In Table 4, series are collected that report on combination therapy of surgery with complete hemithoracic irradiation. Sugarbaker et al[67] advocated that adjuvant radiotherapy should be 40 to 45 Gy to the entire hemithorax, with a 5- to 5.5-Gy boost to areas at high risk for recurrence. Doses limiting thoracic structures are spinal cord (45 Gy), heart (45 Gy), and lung (20 Gy).[68] Hemithoracic radiotherapy equals a total loss of lung function.[69] A shift of the abdominal viscera into the inferior hemithorax after a pneumonectomy limits the safe dose to 30 Gy in the inferior area.[67]

The technique of EPP combined with hemithoracic radiation and systemic chemotherapy was described by Grondin and Sugarbaker.[70] The largest series was described by Sugarbaker et al[46] with 183 patients. The mortality rate was 3.8%. The morbidity rate was 50%, including cardiac arrest, respiratory failure, ARDS, sepsis, contralateral pneumothorax, arrhythmias, pulmonary embolism, empyema, and GI hemorrhage.[46] The median survival in this patient group was 19 months, with a 2-year survival of 38%. In selected patients with the epithelial cell type and without mediastinal nodal metastases at resection, Sugarbaker et al[67] reported a 5-year survival of 45%. Despite aggressive local treatment including pericardium and diaphragm resection, the site of failure was in most instances the ipsilateral hemithorax (35%) followed by the abdomen (26%), the contralateral hemithorax (17%), and other distant sites (8%).[50]

The application of brachytherapy after pleurectomy was studied in 41 patients by Hilaris et al.[44] Measurable gross residual tumor was treated with permanent iodine 125 implantation and residual diffuse disease by temporary iridium 92 implantation or postoperative instillation of phosphorus 32. After this treatment, external radiotherapy on the hemithorax was administered (45 Gy). There was no mortality. Complications occurred in six patients (15%), including one case of radiation pneumonitis and one case of pericarditis. The median survival was 21 months, with a 2-year survival of 40%. At time of the report, 71% of the patients had relapsed. Local recurrence occurred in one third of the relapsed patients, and distant metastasis with or without local recurrence occurred in the other two thirds.[44] An update, including the same patient cohort with larger follow-up, by Mychalczak et al[71] could not confirm this treatment outcome; in this abstract, a median survival of 13 months was reported.

Alberts et al[53] studied the combination of decor-

tication, followed by systemic hemithoracic radiotherapy and systemic chemotherapy. Twenty-six patients were treated. The median survival was 10.9 months. Different combination of treatment modalities did not influence survival.[53]

Another study performed by Mattson et al,[54] with 100 patients included, showed a median survival of 8 months and a 2-year survival of 20%. Five different radiotherapy and chemotherapy regimens were used, but no statistical differences were seen between the groups.[54]

The combination of pleurectomy, external radiotherapy, and systemic chemotherapy was also studied in Memorial Sloan-Kettering Cancer Institute.[52,72] This multimodality treatment resulted in a median survival of 21s month for epithelial mesothelioma and 11 months for fibrosarcomatous mesothelioma.[72]

In a more recent study, Rusch et al[55] reported results of hemithoracic radiotherapy after complete resection in 61 patients. Adjuvant radiotherapy at a median of 54 Gy was well tolerated, except for one esophageal fistula. Only 13% patients had a local recurrence. Distant metastases were seen in 70% of the patients. The median survival was 17 months, and a 3-year survival of 27% is described. For stage I/II, the median survival was 34 months. Based on these results, the group of Rusch et al[55] adapted this treatment regimen as standard treatment for patients with limited pleural mesothelioma.

Surgery and Emphasis on Systemic Chemotherapy

Huncharek et al[57] studied the combination of surgery with postoperative systemic chemotherapy (Table 4). The combination of chemotherapy consisted of cisplatin and doxorubicin or cisplatin and mitomycin C. The median survival was 21 months with a 2-year survival of 23.9%.[54]

A less favorable outcome was found by Ceresoli et al.[43] In this small series (16 patients), the chemotherapy was mostly cisplatin, doxorubicin, or a combination of these agents. The median survival was 14 months.[43]

Hastürk et al[58] treated 20 patients with pleurectomy followed by systemic chemotherapy (cisplatin and mitomycin C) and immunotherapy (α-interferon). This resulted in a median survival of 12 months and a 2-year survival of 15%. The survival was calculated from the onset of chemotherapy.[58]

DaValle et al[56] reported a median survival of 17.5 months. Adjuvant therapy consisted of doxorubicin alone or in combination with other agents or irradiation. The reported survival was no better than that of the 13 patients not receiving adjunctive therapy. This study was not a randomized controlled one.[56]

Surgery and Emphasis on Intrapleural Chemotherapy

Intracavitary chemotherapy has the advantage of high local concentrations of the cytostatic drug while having limited systemic side effects.[73–75] Only direct cytotoxic agents appear rational. The pharmacokinetics of cisplatin and mitomycin are advantageous, but also show significant and sustained plasma levels.[74–76] One of the limiting factors is that the penetration depth of chemotherapy is limited to a few millimeters.[3] Therefore, intrapleural chemotherapy can only be profitable if it is preceded by optimal cytoreduction.

In a study performed by Lee et al[62] with intrapleural cisplatin and cytosine arabinoside after incomplete surgery (pleurectomy/decortication), the median survival was 11.5 months. Rusch et al,[60] Colleoni et al,[63] Sauter et al,[61] and Rice et al[59] studied the use of intrapleural chemotherapy after complete cytoreduction. All patients in these studies received adjuvant systemic chemotherapy. Rusch et al[60] studied the effect of instillation with cisplatin and mitomycin after pleurectomy or decortication. The median survival was 18 months, with a 2-year survival of 40%. The mortality was 3.7%, and significant morbidity was observed in 55%. Chemotherapy-related nephrotoxicity was seen in three patients (11%).[60] Recurrences were seen in 17 of 27 treated patients (63%). All recurrences, except one, were ipsilaterally localized.[51] Colleoni et al[63] applied cisplatin and cytarabine as intrapleural instillation after pleurectomy in 20 patients. One patient had a grade IV nephrotoxicity requiring dialysis. The overall median survival was 11.5 months; patients with minimal residual disease after pleurectomy had a median survival of 24.5 months.[63] In the study of Sauter et al,[61] 13 patients received subtotal pleurectomy followed by intrapleural cisplatin and arabinosylcytosine, resulting in a median survival of 9 months with a 2-year survival of 25%. Rice et al[59] studied 19 patients with stage I MPM undergoing EPP or pleurectomy followed by postoperative intrapleural cisplatin and mitomycin. Grade I/II hematologic toxicity was seen in seven patients (58%). Mild ototoxicity was noticed in one patient. The mortality was 5%. Complications requiring reoperation developed in 16% of the patients. The median survival was 13 months. The site of recurrence was local (58%), distant (17%), or both local and distant (25%).[59]

Hyperthermia itself is cytotoxic; it enhances the cytotoxic effect of the cytostatic drugs, and it stimulates the penetration depth.[77–81] Carry et al[82] studied the addition of hyperthermia to surgery and intrapleural chemotherapy. Three patients with MPM stage I were included in this study. After pleurec-

tomy, an intrapleural perfusion with mitomycin C was performed during 60 min. Because the risk of pulmonary edema is present at temperatures > 43°C, the maximal pleural temperature was 42.6°C.[83] The technique was considered safe and feasible. No systemic toxic levels of mitomycin C were found. Two patients died after 4 months and 11 months, respectively, and one patient survived at least 22 months.[82] Yellin et al[84] treated seven patients with mesothelioma. A combination of surgery and intraoperative hyperthermic pleural perfusion with cisplatin over 60 min was used. The technique was feasible, easy to perform, and relatively safe. A median survival of 15 months was reported, with two patients surviving > 30 months.[84]

A multimodality therapy including surgery, pleural space perfusion with cisplatin, hyperthermia, and postoperative radiotherapy was studied by Ratto et al.[85] The duration of perfusion was 60 min in this study. Radiotherapy (55 Gy) was administered to chest wall incisions. Ten procedures were without any death or toxicity. Ratto et al[85] found higher systemic drug concentrations after pleurectomy/decortication than after pleuropneumonectomy, indicating that the lung plays an important role in cisplatin absorption from the pleural space. Normothermic pleural space perfusion was performed in three patients. The local tissue/perfusate ratio of platinum concentrations tended to be higher after hyperthermic perfusion rather than normothermic perfusion.[85]

In the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, we studied patients with MPM stage I treated with cytoreduction and intraoperative hyperthermic intrathoracic chemotherapy.[86] Cisplatin and doxorubicin were perfused over 90 min under mild hyperthermic conditions (40°C to 41°C). Doxorubicin was chosen because its enhanced activity under hyperthermic conditions; however, the penetration depth is limited.[87,88] Radiotherapy (8 Gy three times) on the thoracotomy scar and drainage tracts was administered to prevent scar recurrences.[4] The treatment was feasible but was accompanied by considerable toxicity. In a report of 11 patients, a median survival of 8 months was found. Disease recurred in three patients after 4 months, 6 months, and 7 months, respectively. The longest survivor without disease was 8 months.[86] An update of our results in 20 patients showed a median survival of 11 months (unpublished data).

Surgery and Emphasis on Intrapleural Photodynamic Therapy

Photodynamic therapy (PDT) has been considered a new mode of adjuvant treatment to sterilize the surgical field. After systemic administration of a photosensitizer, (tumor) cell kill can be achieved by illuminating the resection field with laser light. This principle was first tested by Takita and Dougherty,[89] who used a first-generation photosensitizer (Photofrin; Quadra Logic Technologies; Vancouver, BC). He treated 31 patients and reported a median survival of 12 months. The estimated median survival increased to 21 months when subdivided for stage I/II. Both pleurectomy and EPP were performed to achieve optimal cytoreduction. The mortality was 6.5%. Serious complications were observed in 48.3%, consisting of infection, bronchopleural fistula, cardiac arrhythmia, prolonged ventilatory support, chylothorax, hematothorax, and spontaneous rupture of the spleen.[89] A long-term report of the same institute including 40 patients revealed a median survival of 15 months. In stage I/II, the median survival was 36 months.[65]

An important randomized controlled study using PDT was performed by Pass et al.[64] Photofrin, a first-generation photosensitizer with a long illumination time, was used. Forty-eight patients underwent debulking to, at most, 5-mm residue. He found no survival benefit or improved local control for patients undergoing EPP pleurectomy combined with PDT.[64] The median survival was 14 months. The mortality and morbidity in this study were considerable: 2.1% and 20.8%, respectively. Complications such as death, bronchopleural fistula, esophageal perforation, and empyema are frequently seen when using PDT.[15,64]

Baas et al[90] studied intraoperative PDT after EPP in five patients using a second-generation photosensitizer (meta-tetrahydroxyphenylchlorin). The feasibility study was promising, but in the extended phase I/II study of 28 patients, the median survival was only 10 months.[66,90] In this study, three patients died in the perioperative period; one death was directly related to inappropriately delivered PDT, and two patients with advanced cases died as a result of cardiac complications. The considerable morbidity and mortality preclude this setup for widespread use.[66]

Escalating the light dose, improvement of light delivery, and addition of chemotherapy and radiotherapy are currently being investigated. Distant tumor spread is not prevented by this combined treatment modality.[91]

Discussion

Prospects for the Future

Reviewing the literature on treatment of MPM is not encouraging. Not only has little progress has

been made in the treatment of this disease, it is also clear that very few systematic attempts have been made to evaluate the effects of treatment strategies. Almost without exception, reports are retrospective, with poorly defined patient groups and large variations in treatment schedules. Most reported studies can at best be classified as phase I type feasibility studies. There are very few properly structured phase II studies and no phase III studies at all, in which a treatment schedule has been randomly compared to no treatment. In this era of evidence-based medicine, we can only conclude that no evidence exists of proven effectiveness of any treatment in MPM.

What lessons can be learned from the accumulated experience? The staging of MPM remains difficult by any standard. A preoperative CT scan and mediastinoscopy seem at present to be the minimum requirements for adequate staging. The distinction between stage I and higher stages is often possible. The distinction within stage I according to the IMIG staging system, which is meant to determine operability, is far more difficult.[24] Anyone engaged in surgery for MPM is impressed by the variation of growth characteristics in different patients. Sometimes, the tumor has a clear sharp margin and can easily be separated from neighboring structures; at other times, infiltrative growth with accompanying fibrosis is so dense that any attempt on removal is an illusion. In the present staging system, these characteristics are not fully represented, but determine to a large extent the completeness of any surgery, be it decortication or pleuropneumonectomy. It seems evident that only patients with stage I MPM are candidates who could benefit from aggressive locoregional therapies. However, it is clear that this has not been the case in most of the presently reviewed studies.

In this review, we could not find clear arguments to choose between decortication and pleuropneumonectomy as a first-choice surgical strategy. In many cases, decortication is not feasible because involvement of lung parenchyma. When technically possible, decortication seems to result in roughly the same survival as does pleuropneumonectomy (or no treatment?), but operative mortality is slightly decreased. In this review, we have focused on several multimodality treatments. Surgery combined with external radiotherapy included the whole hemithorax as radiation field in contrast to those in which only the surgical scars were radiated. In selected patients, a clear survival benefit is found; however, when critically analyzed, only 1 to 2% of all patients with mesothelioma could benefit of this treatment.[18,67] Although differences are limited, there remains an impression that survival in the series with external radiotherapy is somewhat longer than in the series not including hemithorax radiotherapy (approximately 20-month median survival in recent reports[32,55] vs approximately 15-month median survival in other combination therapies[43,56–66]). Side effects of radiotherapy on the liver and heart are mentioned but not quantified, especially not in the long term.

Autopsy studies of patients with MPM revealed that more than one half of the patients had disseminated MPM.[18] Therefore, systemic chemotherapy seems to be a prerequisite, but the survival of series with the combination of surgery with systemic chemotherapy appears very similar to the surgery-alone series.[43,56–58] The same is valid for the studies on the combination of surgery with intrapleural chemotherapy.[59–63] The intrapleural chemotherapy approach has probably not yet shown its full potential, as only few drugs (doxorubicin and cisplatin) have been studied, and dosage can probably still be increased. The combination of surgery with PDT has not shown a clear improvement of median survival until now. Furthermore, physical aspects like dosimetry of the light makes general application of this treatment difficult. PDT as part of a multimodality approach cannot be recommended at this stage.[64–66]

The fact remains that the large majority of patients with MPM die of locoregional failure despite aggressive locoregional therapy. This is especially true if recurrences in adjacent cavities (pericardium, contralateral pleura, and abdomen) are considered as regional failure, as we believe they should. The high locoregional failure could be explained by the relative insensibility of MPM to radiotherapy and chemotherapy. Intensifying the therapy is limited by the intolerance of adjacent vital structures (especially the lung).[34]

The conclusion of this review can only be that at this moment no therapy has been adequately shown to have any proven benefit in the treatment of MPM. At this moment, the combination of complete surgery, being decortication or pleuropneumonectomy, in combination with hemithorax radiotherapy seems promising only in selected patients. Intrapleural hyperthermic chemotherapy clearly needs a better-designed study. Future adjuvant therapies will also focus on gene therapy, small molecules (like tyrosine kinase inhibitors), and angiogenesis inhibitors.[15] For gene therapy, however, results have been disappointing given the remarkable results in animals.[92] Future studies would provide more useful information if they used a randomized phase II design, comparing the defined treatment with a no-treatment arm, especially if this would involve a quality-of-life assessment.

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